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[54] MACROCYCLIC CHELATING AGENTS AND CHELATES THEREOF

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[30] Foreign Application Priority Data

Dec. 24, 1987 [IT] Italy 232176 A/87

[51] Int. Cl.[5] C07F 5/00; A61K 49/00

[52] U.S. Cl. 534/10; 534/14; 534/15; 534/16; 540/450; 540/465; 540/452; 540/475; 424/1.1; 556/40; 556/44; 556/50; 556/56; 556/63; 556/77; 556/116; 556/125; 556/134; 556/136; 556/141; 556/148; 556/62; 556/115; 556/55; 556/133

[58] Field of Search 534/16, 15, 10, 14; 540/450, 452, 465, 474; 424/1.1; 556/40, 44, 50, 55, 56, 62, 63, 77, 115, 116, 125, 134, 133, 136, 141, 148

[56] References Cited

U.S. PATENT DOCUMENTS

4,639,365	1/1987	Sherry	424/9
4,885,363	12/1989	Tweedle et al.	534/16 X
4,994,560	2/1991	Kruper, Jr. et al.	534/10
5,006,643	4/1991	Fazio et al.	534/10
5,049,667	9/1991	Schaefer et al.	540/474

FOREIGN PATENT DOCUMENTS

0255471	7/1987	European Pat. Off. .
8901476	2/1989	PCT Int'l Appl. .

OTHER PUBLICATIONS

Desreux et al., "Highly Stable Lanthanide Macrocyclic Complexes", Nucl. Med. Biol. vol. 15, pp. 9-15, 1988.

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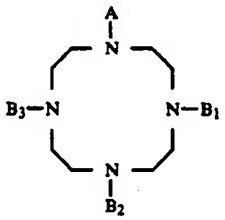
[57] ABSTRACT

Macrocyclic derivatives of 1,4,7,10-tetraazacyclododecane of general formula (I) hereinbelow, wherein A is a group of formula (II) hereinbelow, in which R is H or alkyl or optionally substituted benzyl or a $H(OCH_2CH_2)_{1-4}$-, $Me(OCH_2CH_2)_{1-4}$-, or $Et-(OCH_2CH_2)_{1-4}$-group, X or O—R_1, in which R_1 is H or alkyl, hydroxyalkyl, alkoxyalkyl, alkoxyhydroxyalkyl or a polyoxaalkyl group or X is —NR_2R_3, in which R_2 and R_3 are H or alkyl, hydroxyalkyl, alkoxyalkyl or alkoxyhydroxyalkyl, and B_1, B_2 and B_3 have the same meanings as A or are H or a group of formula (III) hereinbelow, in which R_4 is H or alkyl, Y is a O—R_5 group, wherein R_5 is H or alkyl, hydroxyalkyl, alkoxyalkyl, alkoxyhydroxyalkyl or a polyoxaalkyl group, or Y is a —NR_6R_7 group, wherein R_6 and R_7 are H or alkyl, hydroxyalkyl, alkoxyalkyl, or alkoxyhydroxyalkyl, said derivatives optionally being salified, and the complex salts thereof, are used as pharmaceuticals and/or diagnosic agents.



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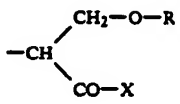

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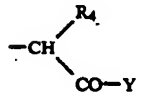


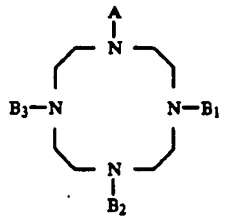
(III)

18 Claims, No Drawings

MACROCYCLIC CHELATING AGENTS AND CHELATES THEREOF

This application is the U.S. national phase of PCT Application No. PCT/EP88/01166 filed Dec. 16, 1988 which was based on Italian Patent Application No. 23217 A/87 which was filed on Dec. 24, 1987 and is also a Continuation-in-Part Application of U.S. Ser. No. 002,115 which was filed Jan. 12, 1987, now U.S. Pat. No. 4,916,246.

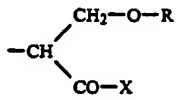
The present invention relates to novel macrocyclic chelating agents deriving from 1,4,7,10-tetraazacyclododecane of general formula I



I

wherein

A is a group of formula

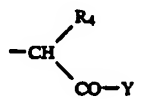


in which

R is H or a C_1–C_5 straight or branched alkyl group, or a benzyl group which can be mono- or polysubstituted on the aromatic ring by halogen, hydroxy, carboxy, carbamoyl, alkoxycarbonyl, sulphamoyl, lower alkyl, lower hydroxyalkyl, amino, acylamino, acyl, hydroxyacyl groups, or a group of formula $H(OCH_2CH_2)_{1-4}$-, $Me(OCH_2CH_2)_{1-4}$-, or $Et(OCH_2CH_2)_{1-4}$-,

X is a O—R_1 group in which R_1 is H or a C_1–C_5 alkyl, hydroxyalkyl, alkoxyalkyl, alkoxyhydroxyalkyl group, or a polyoxaalkyl group having 1 to 15 oxygen atoms and 3 to 45 carbon atoms, or X is a —NR_2R_3 group in which R_2 and R_3, which can be the same or different, are C_1–C_6 alkyl, hydroxyalkyl, alkoxyalkyl or alkoxyhydroxyalkyl groups having up to 5 hydroxy groups and

B_1, B_2 and B_3, which can be the same or different, have the same meaning as A or they are H or a group of formula



in which

R_4 is H or a C_1–C_5 straight or branched alkyl group,

Y is a O—R_5 group in which R_5 is H or a C_1–C_5 alkyl, hydroxyalkyl, alkoxyalkyl, alkoxyhydroxyalkyl group, or a polyoxaalkyl group having 1 to 15 oxygen atoms and 3 to 45 carbon atoms, or Y is a —NR_6R_7 group in which R_6 and R_7, which can be the same or different, are H or C_1–C_6 alkyl, hydroxyalkyl, alkoxyalkyl or alkoxyhydroxyalkyl groups having up to 5 hydroxy groups,

said derivatives being, if necessary, salified with suitable organic or inorganic bases,

and the complex salts of the abovesaid chelating agents with suitable metal ions in the acid, basic or neutral form or, if necessary, neutralized with inorganic or organic acid or base ions, and eventually chemically conjugated with macromolecules or incorporated in suitable carriers.

The present invention also relates to the preparation of compounds of general formula I and of the complex salts thereof, to their uses and, when indicated, to the pharmaceutical and diagnostic compositions thereof.

The chelating compounds of the present invention and the complex salts thereof can have a wide range of applications. No limiting examples of use of said chelating agents are the recovery, separation, selective extraction of metal ions even at very low concentrations, their therapeutical use as detoxifying agents in cases of inadvertent bodily incorporation of metals or radioisotopes, their use as ion carriers, or the other ones apparent to those skilled in the art. In such uses the chelating agents may be used directly or often they have been bonded covalently or non-covalently to macromolecules or insoluble surfaces or have been otherwise incorporated into structures that can carry them to specific sites.

In particular the complex salts of the chelating agents of formula I with the metal ions of the elements with atomic numbers of 20 to 31, 39, 42, 43, 44, 49 or 57 to 83 and, optionally, salified by physiologically biocompatible ions of organic or inorganic acids or organic or inorganic bases or aminoacids, are surprisingly suitable for use as contrast agents in medical diagnosis in nuclear medicine and in N.M.R., E.S.R., X-ray and/or ultrasonic diagnosis. Said derivatives, for the purpose of optimal diagnostic use, can also be bound or incorporated covalently or non-covalently into biomolecules, macromolecules or molecular aggregates characterized in that they can selectively concentrate in the organ or in the tissue under examination.

The imaging of internal structures of living subjects is becoming more and more relevant in medical diagnosis.

Among the most recent techniques, the use of radioisotopes as internal tracers in the organism should be mentioned. One of the biggest problems connected with the use of radioisotopes is their selectivity of distribution, while another important aspect is their excretion in an acceptable time.

Another imaging technique concerns with the use of ultrasounds to measure the difference in the reflections at the interfaces between tissues of different density. The administration of a suitable amount of a dense non-radioactive element or metal ion can give such a difference in reflectivity that can emphasize even small otherwise non detectable lesions.

A third diagnostic technique uses nuclear magnetic resonance to create internal images of the human body. In this field, the development of contrast agents is of particular importance for the following reasons:

a) to improve the specificity of the diagnosis,

b) to identify at an earlier stage small lesions,

c) to more precisely define the extension of a tumoral mass,

d) to improve the signal to noise ratio and to shorten the time of acquisition of the images, allowing also better use of the instruments,

e) to increase the contrast between those contiguous areas (for instance abdominal or pelvic) where it is particularly difficult to obtain well defined images,

f) to obtain good informations on blood flow and on tissue perfusion.

As far as regards N.M.R. diagnosis, contrast media containing paramagnetic complex salts of lanthanides and transition metals have already been claimed for instance in patents EP 71564, U.S. Pat. Nos. 4,647,447, 4,687,658, 4,639,365, 4,678,667 and in patent applications DE 3401052, EP-A 135125, EP-A 130934, DE 3324236, EP-A 124766, EP-A 165728, WO 87/02893, EP-A 230893, EP-A 255471, EP-A 232751, EP-A 292689, EP-A 287465, WO 87/06229, WO 89/01475, WO 89/01476.

However all the till now developed contrast agents for N.M.R. present some problems as far as regards their capacity of influencing the relaxation time of the atomic nuclei involved, their often insufficient selectivity in bonding the metal ion, their stability, their selectivity for the organ under examination, or their biological tolerability. The tendency of many complexes to exchange the central metal ion with trace-metals essential to the organism or with ions, for example $Ca^{(2+)}$, which in vivo are present in relatively great amounts (see on the subject P.M. May "The present status of chelating agents in Medicine", page 233), further limits their possibilities of use. In fact, in case of insufficient stability of the complex, the organism may be deprived of trace-metals of vital importance and receive undesired and toxic heavy metals such as Gd, Eu or Dy. Although it is true that the toxicity of the complex is often, but not always, lower than the one of the free paramagnetic ion, it is also true that the complexation usually brings a decrease of the magnetic relaxation efficacy, responsible for some contrasting effects.

Several unsolved problems in connection with an optimal contrast agent therefore still remain, chiefly concerning: a strong effect on the relaxation time of the relevant nuclei, a high stability of the complex both in solution and in the organism, an adequate water solubility, a specificity of distribution in the various parts of the organism, a suitable rate of elimination from the involved organ and tissue.

One of the most studied paramagnetic metal ions is $Gd^{(3+)}$, in particular when complexed with the chelating agent diethylenetriamino-pentaacetic acid (DTPA) (Runge et al. (1983) Am. J. Radiol. V 141, p. 1209 and Weinman et al. (1984) Am. J. Radiol. V 142, p. 619). Said complex salified with D(−)N-methyl-glucamine is considered at the moment one of the most satisfying from the point of view of activity, toxicity and of its use in general.

However, in spite of these positive features, this compound cannot yet be considered fully respondent to the characteristics of an optimal contrast agent for various reasons, among which for instance the fact that Gd-DTPA/N-Methyl-D-glucamine is too quickly removed from the blood stream and from the lesions of the tissues under examination. This reduces the time available for obtaining images significant from diagnostic point of view. Moreover the diffusion of the contrast agent between the healthy part and the diseased one is often so fast that the contrast between the two regions can be too weak.

To overcome these difficulties, the problem has been approached in many ways among which the most interesting are:

a) Other chelating agents have been studied, in particular macrocyclic ones, of which the most effective proved to be 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). However its complexes continue to present problems analogous to the ones of DTPA.

b) Gadolinium and its chelating agents have been chemically conjugated to macromolecules such as, for instance, proteins (albumin, etc.), immunoglobulins, or to cellulose or other polymeric matrices. On the one hand this generally improved the relaxivity of Gd, but on the other hand it was necessarily accompanied by a sub-optimal dosage, because of limitations in solubility, toxicity and the substitution density of the macromolecules. Furthermore, when one of the ligand sites of the chelating agents is used to form the chemical bond with the macromolecule, there is also normally a reduction in the stability of the resulting complex.

The chelating agents of formula I have shown an excellent scavenging capacity for metal ions even in very diluted solutions. A significant example of said property is the capacity to capture the $Cu^{(2+)}$ ion from its aqueous solutions by 2-[1,4,7,10-tetraaza-4,7,10-tri(-carboxymethyl)-cyclododeca-ne-1-yl]-3-benzyloxypropionic acid, the method of synthesis of which is reported in examples 2 and 3.

With regards to their use in diagnosis, metal complexes with the chelating agents object of the present invention have proved surprisingly satisfying for instance with respect to the requirements for an N.M.R. contrast agent. Among the complexes particular importance is to be given to the complexes of $Gd^{(3+)}$, which distinguish themselves for excellent stability, relaxivity and selectivity for the organ or tissue under examination.

These compounds have a wide field of application, allowing administration by intravasal route (for example intravenous, intraarterial, intracoronaric, intraventricular, etc.), as well as intrathecal, intraperitoneal, intralymphatical, intracavital and intraparenchymal routes. Both the soluble and the poorly soluble compounds are suitable for oral or enteral administration, and therefore of particular usefulness for visualization of the gastrointestinal tract. For parenteral administration they are preferably formulated as a sterile aqueous suspension or solution, whose pH can range for instance from 6.0 to 8.5. Said sterile aqueous suspensions or solutions can be administered in concentrations varying from 0.002 to 1.0 molar.

Said formulations, can also be lyophilized and supplied as such, to be reconstituted at the moment of their use. For gastrointestinal use or for injection in body cavities said agents can be formulated as a suspension or a solution containing additives suitable for instance to increase viscosity.

For oral administration they can be formulated according to preparation methods commonly used in pharmaceutical technology, optionally also as a coated formulation so as to have additional protection against the acid pH of the stomach, preventing in that way the release of the chelated metal ions which takes place in particular at the pH values typical of gastric juices. Other excipients, such as sweetening or flavouring

agents, can be added according to known pharmaceutical formulation techniques.

Suspensions or solutions of complex salts can also be formulated as aerosols to be used in aerosol-bronchography.

Some of the complex compounds of the invention have a surprising organ specificity, in that they particularly concentrate in the liver, in the spleen or, after intralymphatic, intraparenchymal, intramuscular or subcutaneous application, in the lymphatic vases and in the lymph nodes. The resulting contrast between the organ under examination and adjacent tissues permits improved imaging of said organ by N.M.R.

With regard to their use in diagnosis, metal complexes of the chelating agents object of the present invention can also be used as contrast agents in nuclear medicine and for electron spin resonance or echographic analyses.

In these cases however the metal central ions in the chelated complexes are, respectively, a radioisotope for example ^{51}Cr, ^{68}Ga, ^{111}In, ^{99m}Tc, ^{140}La, ^{168}Yb or a non-radioisotope able to alter, owing to the density of its solutions, the speed of the ultrasonic waves transmitted and reflected.

In the compounds of general formula I, A is preferably a β-hydroxy-α-propionic, β-methoxy-α-propionic or β-benzyloxy-α-propionic group, optionally esterified or preferably substituted by an amide residue which can be free, mono-or bi-substituted by alkyl, hydroxyalkyl, alkoxyalkyl or alkoxyhydroxyalkyl groups.

R can preferably be H or a straight or branched alkyl group, such as a methyl, ethyl, propyl, isopropyl, butyl, isobutyl group or a benzyl or a substituted benzyl group as defined in formula I.

R can also be an acyl or hydroxyacyl group.

R can also be a polyoxaethylene group of formula $H(OHCH_2CH_2)_{2-4}$-, $Me(OCH_2CH_2)_{2-4}$-, o $Et-(OCH_2CH_2)_{2-4}$-.

X can be a hydroxy group or also a —O—R_1 group, wherein R_1 is as defined in formula I.

Non-limiting examples of R_1 are the following: methyl, ethyl, isopropyl, 2-hydroxyethyl, 2-hydroxypropyl, 1,3-dihydroxyisopropyl, polyoxaalkyl groups.

X can preferably be also an hydroxyalkylamino group of formula —NR_2R_3, in which R_2 and R_3 are as defined in formula I. Non-limiting examples of said groups are the following ones:

amino-, 2-hydroxyethylamino-, 2-hydroxypropylamino-, 2,3-dihydroxypropylamino-, 1,3-dihydroxyisopropylamino-, 1,3-dihydroxy-2-methyl-isopropylamino-, 2,3,4-trihydroxy-1-butylamino-, 1,3,4-trihydroxy-2-butylamino-, 1,3-dihydroxy-2-hydroxymethyl-isopropylamino-, N-methyl-N-2-hydroxyethylamino-, N-methyl-N-2,3-dihydroxypropylamino-, N-methyl-N-1,3-dihydroxyisopropylamino-, N-methyl-N-2,3,4,5,6-pentahydroxyhexylamino-, N-2-hydroxyethyl-N-2,3-dihydroxypropylamino-, N-2-hydroxyethyl-N-1,3-dihydroxyisopropylamino-, N,N-bis-(2-hydroxyethyl)amino-, N,N-bis(2,3-dihydroxypropyl-)amino-, N,N-bis-(1,3-dihydroxyisopropyl)amino groups.

In compounds of general formula I, the B_1, B_2, B_3 groups preferably are an acetic or an α-propionic group, eventually esterified or substituted by an amido residue which can be in the free form or mono- or bi-substituted by alkyl, hydroxyalkyl, alkoxyalkyl or alkoxyhydroxyalkyl groups.

R_4 can preferably be hydrogen or straight or branched lower alkyl, preferably methyl.

Non-limiting examples for R_4 are the following: hydrogen, methyl, straight or branched propyl, butyl and pentyl groups, as defined in formula I.

Y can preferably be a hydroxy group or a —O—R_5 group, in which R_5 has the above defined meanings of formula I.

Non limiting examples of R_5 are the following: methyl, ethyl, isopropyl, 2-hydroxyethyl, 2-hydroxypropyl, 1,3-dihydroxyisopropyl, polyoxaalkyl groups.

Y can preferably be also a hydroxyalkylamino group of formula —NR_6R_7 in which R_6 and R_7 have the above mentioned meanings of formula I.

Non-limiting examples of said groups are the following ones:

amino, 2-hydroxyethylamino-, 2-hydroxypropylamino-, 2,3-dihydroxypropylamino-1,3-dihydroxyisopropylamino-, 1,3-dihydroxy-2-methyl-isopropylamino-, 2,3,4-trihydroxy-1-butylamino-, 1,3,4-trihydroxy-2-butylamino-, 1,3-dihydroxy-2-hydroxymethyl-isopropylamino-, N-methyl-N-2-hydroxyethylamino-, N-methyl-N-2,3-dihydroxypropylamino-, N-methyl N-1,3-dihydroxyisopropylamino-, N-methyl-N-2,3,4,5,6-pentahydroxyhexylamino-, N-2-hydroxyethyl-N-2,3-dihydroxypropylamino-, N-2-hydroxyethyl-N-1,3-dihydroxyisopropylamino-, N,N-bis-(2-hydroxyethyl)amino-, N,N-bis-(2,3-dihydroxypropyl-)amino-, N,N-bis-(1,3-dihydroxyisopropyl)amino groups.

Metal ions suited to form complex salts with the chelating agents of general formula I are mainly the di- or trivalent ions of the elements having atomic numbers ranging from 20 to 31, 39, 42, 43, 44, 49, or from 57 to 83 and particularly preferred are $Fe^{(2+)}$, $Fe^{(3+)}$, $Cu^{(2+)}$, $Cr^{(3+)}$, $Gd^{(3+)}$, $Eu^{(3+)}$, $Dy^{(3+)}$ or $Mn^{(2+)}$.

Among the metal radioisotopes, particularly preferred are ^{51}Cr, ^{68}Ga, ^{111}In, ^{99m}Tc, ^{140}La, ^{168}Yb.

Preferred inorganic acid anions comprise ions such as chlorides, bromides, iodides or other ions such as sulfate. Preferred organic acid anions comprise ions of acids which are generally pharmaceutically used to salify basic substances, such as acetate, succinate, citrate, fumarate, maleate.

Preferred inorganic base cations comprise alkali metal ions, such as lithium, potassium and sodium, the latter being particularly preferred.

Preferred organic base cations comprise primary, secondary and tertiary amines, such as ethanolamine, diethanolamine, morpholine, glucamine, N,N-dimethylglucamine and N-methylglucamine, the latter being preferred.

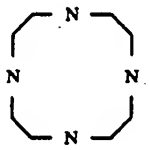
Preferred amino acid cations comprise, for example, those of lysine, arginine and ornithine.

Non-limiting examples of the macromolecules suited for conjugation with the chelate complexes of the invention are the following: biomolecules, such as hormones (insulin), prostaglandins, steroidal hormones, amino sugars, peptides, proteins (albumin, human serum albumin), lipids, antibodies such as monoclonal antibodies, polysaccharide chains.

The chelated complexes of the invention can also be incorporated into liposomes, used in form of mono- or multi-lamellar vescicles.

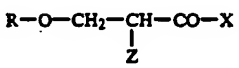
The chelating agents of general formula I and the complex salts thereof are preferably prepared by reacting 1,4,7,10-tetraazacyclododecane (II), prepared ac-

cording to the method of Atkins et al. (JACS 96, 2268 (1974)),



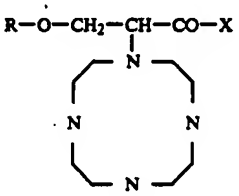
II

with the desired α-halo-propionyl derivative III



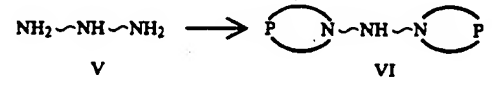
III

wherein Z is halogen and R and X have the above defined meanings, to give the addition product IV



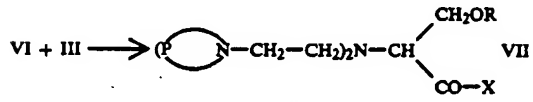
IV

or the corresponding polysubstituted products on the nitrogen atoms of II, depending on the excess of III used. Compound IV can also be obtained, for example, by protecting diethylenetriamine V with a suitable protecting group P, wherein P can be, for example, a phthaloyl group or another appropriate protective group known in the literature (T. W. Greene: "Protective groups in organic synthesis"—1980),



V VI

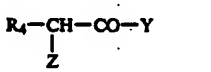
by alkylating the resulting compound VI with the proper halo-derivative III



VII

and by finally condensing the resulting product VII, after deprotection and subsequent tosylation, with tosyldiethanolamine.

Compound IV or the polysubstituted analogues thereof can in turn be subjected to condensation with the appropriate α-halo-acetic derivative VIII, or with a suitable precursor thereof (such as an ester or a nitrile),



VIII

wherein Z is halogen and R_4 and Y have the above defined meanings, to give the desired chelating agents of general formula I.

Finally, chelation of the desired metal ion is obtained preferably by reacting the appropriate derivative of formula I with the stoichiometric amount of metal, in form of a salt or an oxide, possibly in the presence of the base or acid amounts necessary for neutralization.

Condensation of II with III is carried out preferably in water or in a dipolar aprotic organic solvent, such as dimethylformamide (DMF) or dimethylacetamide (DMAC) or in a mixture thereof, at a temperature from 30° to 150° C., preferably from 40° to 100° C.

Subsequent condensation of IV with VIII can be effected in an aqueous medium or in an organic solvent, in the presence of an appropriate inorganic or organic base, such as sodium hydroxide, potassium hydroxide, potassium carbonate or tetrabutylammonium hydroxide (TBAOH), at a pH ranging from 8 to 12, preferably from 9 to 11. The temperature can range from 40° to 100° C., preferably from 50° to 70° C.

Finally, preparation of the metal complex salt is preferably carried out in water or in an appropriate water-alcohol mixture, while the temperature can range from 25° to 100° C., preferably from 40° to 80° C.

The choice of the metal ion and, if necessary, of the neutralizing ion is strictly related to the use of the resulting complex.

EXAMPLE 1

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-propionic acid, trihydrochloride

A) Sodium 2-chloro-3-benzyloxypropionate

85 g of 2-chloro-3-benzyloxypropionic acid (0.396 mol) were suspended in 550 ml of water and neutralized to pH 7 with 10% sodium hydroxide. After stirring for 15 min, the resulting aqueous solution was washed with ethyl ether and evaporated to dryness under vacuum to give the desired compound.

90.6 g sodium 2-chloro-3-benzyloxypropionate (0.383 mol) were obtained.

Yield: 96.7%;

Elemental analysis: % calc.: C 50.75; H 4.26; Cl 14.98. % found: C 50.68; H 4.33; Cl 14.89.

B)

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-propionic acid, trihydrochloride

A suspension of 17.2 g of 1,4,7,10-tetraazacyclododecane (0.1 mol) and of 71 g of sodium 2-chloro-3-benzyloxypropionate (0.3 mol) in 70 ml of water was heated to 50° C. for 24 h. The resulting solution was diluted to 400 ml with water, dropped into 200 ml of 2N HCl, extracted several times with methylene chloride and then was evaporated to dryness under vacuum.

The crude residue was taken up into 400 ml of water and adsorbed on amberlite IR 120, from which it was eluted by means of 5N sodium hydroxide. By concentration of the basic eluate 29 g of a residue were obtained, which were dissolved in 400 ml of absolute ethanol at 60° C.; the solution was acidified with 200 ml of 6N HCl/EtOH and the resulting precipitate was stirred at 60° C. for 1 h. After cooling, the solid was filtered and dried to give the desired compound. 33.5 g of 2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxypropionic acid, trihydrochloride (0.0729 mol) were obtained.

Yield: 72.9%; m.p. 221°–224° C.

Titres: (NaOH): 96.9%; ($AgNO_3$): 99.0%.

Elemental analysis: % calc.: C 47.01; H 7.23; Cl 23.13; N 12.18; % found: C 47.13; H 7.32; Cl 22.92; N 12.09;

TLC: Support: silica gel plate (Merck G60)

Eluent: $CHCl_3$: AcOH: H_2O=5:5:1
Developer: Cl_2+o-Toluidine
Rf=0.35
^1H-NMR, ^{13}C-NMR and IR spectra agreed with the indicated structure.

EXAMPLE 2

2-[1,4,7,10-tetraaza-7-(1-carboxy-2-benzyloxy-ethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid

A solution of 12 g of 1,4,7,10-tetraazacyclododecane (0.069 mol) and 82.32 g of sodium 2-chloro-3-benzyloxypropionate (0.348 mol), obtained according to the process described in example 1-A, in 120 ml of DMF was placed in a sealed vessel and heated to 50° C. for 30 h.

The salt formed was filtered and the solvent was distilled off under reduced pressure. The residue was taken up into 300 ml of water, the pH was adjusted to 2.5 with 10% hydrochloric acid, then the mixture was extracted with four 50 ml portions of methylene chloride.

The organic phase was evaporated to dryness and the residue was dissolved in 200 ml of 0.01N HCl and washed with ethyl ether.

The pH was adjusted to 6 with 10% sodium hydroxide and the aqueous solution was evaporated to dryness. The crude residue was taken up into 30 ml of water and adsorbed on amberlite IR 120, from which it was eluted with 5N ammonium hydroxide.

By concentration of the basic eluate, a residue of 7 g was obtained, which was crystallized from water.

5.85 g of 2-[1,4,7,10-tetraaza-7(1-carboxy-2-benzyloxyethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid (0.011 mol) were obtained.

Yield: 16%; m.p.: 170°–175° C.

Elemental analysis: % calc: C 63.61; H 7.63; N 10.60. % found: C 63.48; H 7.82; N 10.51.

Analogously, the following compounds were obtained:

2-[1,4,7,10-tetraaza-4-(1-carboxy-2-benzyloxy-ethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid;

2-[1,4,7,10-tetraaza-4,7-di(1-carboxy-2-benzyloxy-ethyl)cyclododecane-1-yl]-3-benzyloxypropionic acid.

EXAMPLE 3

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid (Method A)

To a suspension of 23 g of 2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxypropionic acid trihydrochloride (0.05 mol), obtained according to the process described in example 1, and 27.8 g of bromoacetic acid (0.2 mol) in 100 ml of water about 60 ml of 6N sodium hydroxide were added, under stirring, to reach pH=10. The mixture was heated to 50° C. for 17 h and the pH was kept at 10 by further additions of 6N sodium hydroxide.

The solution was cooled and applied to amberlite IR 120, from which the product was eluted with 5N ammonium hydroxide. The basic eluate was evaporated to dryness, the resulting crude compound was dissolved in water and the solution was acidified to pH 3 with 5N HCl.

The precipitate was filtered and crystallized from water to give the desired compound.

15.3 g of 2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid (0.029 mol) were obtained.

Yield: 58.4%; m.p.: 173° C. with dec.

Titre: (NaOH): 99.6%; ($ZnSO_4$): 99.5%; (HPLC): 99.0%.

Elemental analysis: % calc: C 54.95; H 6.92; N 10.68. % found: C 54.77; H 6.96; N 10.77.

^1H-NMR, ^{13}C-NMR and IR spectra agreed with the indicated structure.

EXAMPLE 4

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid (Method B)

A mixture of 10 g of 2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxypropionic acid (0.028 mol), obtained according to the process described in example 1 but without formation of the hydrochloride, and 15.57 g of bromoacetic acid (0.112 mol) in 60 ml of water was treated according to the same process as in example 3, to give the desired compound.

7.93 g of 2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid (0.015 mol) were obtained.

Yield: 54%; m.p.: 169°–172° C. with dec.

Titre: (NaOH): 99.3%; ($ZnSO_4$): 99.5%.

Elemental analysis: % calc.: C 54.95; H 6.92; N 10.68. % found: C 54.71; H 7.00; N 10.64.

The other chemico-physical characteristics agreed with the ones of the compound obtained according to method A (example 3).

Analogously, the following compounds were obtained:

2-[1,4,7,10-tetraaza-4,7,10-tri(1-carboxy-ethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid.

2-[1,4,7,10-tetraaza-4-(1-carboxy-2-benzyloxy-ethyl)-7,10-di(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid.

2-[1,4,7,10-tetraaza-7-(1-carboxy-2-benzyloxy-ethyl)-7,10-di(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid.

2-[1,4,7,10-tetraaza-4,7-di(1-carboxy-2-benzyloxy-ethyl)10-carboxymethyl-cyclododecane-1-yl]-3-benzyloxypropionic acid.

EXAMPLE 5

D(−)-N-methylglucamine salt of the $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid complex.

To a suspension of 100 g of 2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid (0.19 mol), obtained according to the process described in example 3, in 150 ml of water 36.6 g of D(−)-N-methylglucamine (0.187 mol) were added. 19.47 g of Gd_2O_3 (0.095 mol) were added to the solution and the resulting suspension was heated to 50° C. for 4 hours. The reaction mixture was filtered and the pH was adjusted to 6.5 by means of a 10% aqueous D(−)-N-methylglucamine solution. The resulting solution was then evaporated and dried to give the desired compound.

159 g of D(−)-N-methylglucamine salt of the $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid complex (0.182 mol) were obtained.

Yield: 95.8%; m.p.: 137° C.

Titre: (HPLC): 99.3%.

Elemental analysis: % calc.: C 42.56; H 5.76; Gd 17.99; N 8.01. % found: C 42.42; H 5.96; Gd 17.63; N 7.92.

$[\alpha]_{365}^{20} = -15.36°$; $[\alpha]_{436}^{20} = -11.22°$; $[\alpha]_{546}^{20} = -6.7°$; $[\alpha]_{589}^{20} = -5.7°$; (C=5% H_2O).

Analogously, the following compounds were obtained:

$Dy^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid salt of D(−)-N-methylglucamine, obtained with Dy_2O_3.

$La^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid salt of D(−)-N-methylglucamine, obtained with La_2O_3.

$Yb^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid salt of D(−)-N-methylglucamine, obtained with Yb_2O_3.

EXAMPLE 6

D(−)-N-methylglucamine salt of $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid complex

A solution of 92 g of D(−)-N-methylglucamine salt of $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid complex (0.105 mol), obtained according to the process described in example 5, in 550 ml of water, to which 153 g of 5% palladium on charcoal had been added, was hydrogenated for 5 h at room temperature.

The catalyst was removed by filtration and the aqueous solution was evaporated under vacuum at 50° C. Upon drying the residue to constant weight, the desired debenzylated compound was obtained.

67 g of D(−)-N-methylglucamine salt of $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid complex (0.084 mol) were obtained.

Yield: 80%; m.p.: 180° C. (dec.).

Elemental analysis: % calc: C 36.77; H 5.65; Gd 20.06; N 8.93. % found: C 36.47; H 5.47; Gd 20.29; N 8.83.

$[\alpha]_{365}^{20} = -16.7°$; $[\alpha]_{436}^{20} = -11.2°$; $[\alpha]_{546}^{20} = -6.7°$; $[\alpha]_{589}^{20} = -5.8°$ (C=5% H_2O).

Analogously, the following compounds were obtained:

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-hydroxypropionic acid.

2-[1,4,7,10-tetraaza-4-(1-carboxy-2-hydroxy-ethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid.

2-[1,4,7,10-tetraaza-7-(1-carboxy-2-hydroxy-ethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid.

2-[1,4,7,10-tetraaza-4,7-di(1-carboxy-2-hydroxy-ethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid.

2-[1,4,7,10-tetraaza-4-(1-carboxy-2-hydroxy-ethyl)-7,10-di(carboxymethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid.

2-[1,4,7,10-tetraaza-7-(1-carboxy-2-hydroxy-ethyl)-4,10-di(carboxymethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid.

2-[1,4,7,10-tetraaza-4,7-di(1-carboxy-2-hydroxy-ethyl)-10-carboxymethyl-cyclododecane-1-yl]-3-hydroxypropionic acid.

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid.

EXAMPLE 7

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide

A) 3-benzyloxy-2-chloropropionylchloride

119 g of thionyl chloride (1 mol) were added dropwise to 107.3 g of 3-benzyloxy-2-chloropropionic acid (0.5 mol) at 30° C. After refluxing the reaction mixture for 2 h, 33 g additional thionyl chloride (0.277 mol) were added and the mixture was refluxed for another 30 min.

Excess thionyl chloride was distilled off under reduced pressure and the desired compound was distilled under vacuum.

95.8 g of 3-benzyloxy-2-chloropropionylchloride (0.41 mol) were obtained.

Yield: 82%; b.p.: 125°–131° C.; 0.05 mbar.

Titre: (reduction with Zn): 99.9%. (Argentometric): 96.0%.

Elemental analysis: % calc: C 51.53%; H 4.32%; Cl 30.42%. % found: C 51.30%; H 4.46%; Cl 29.48%.

^{1}H-NMR, ^{13}C-NMR and IR spectra agreed with the indicated structure.

B)

2-Chloro-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide

A solution of 70 g of 3-benzyloxy-2-chloropropionylchloride (0.3 mol) in 150 ml of tetrahydrofuran was added dropwise during about 2 h to a solution of 32.6 g of 2-amino-1,3-dihydroxyisopropane (0.36 mol) in 150 ml of water and 250 ml of tetrahydrofuran. During the addition of the chloride, the pH of the solution was kept constant at 10 by addition of 6N sodium hydroxide.

To the reaction mixture 250 ml of water were added. Upon concentration to 450 ml a white product precipitated, which was filtered and crystallized from water, after treatment with Carbopuron 4N to give the desired compound.

62.2 g of 2-chloro-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide (0.218 mol) were obtained.

Yield: 72.6%; m.p.: 133°–135° C.

Titre: (Argentometric): 99.8%.

Elemental analysis: % calc: C 54.27; H 6.30; Cl 12.32; N 4.87. % found: C 54.19; H 6.38; Cl 12.24; N 4.84; H_2O 0.22.

HPLC: 99%.

^{1}H-NMR, ^{13}C-NMR and IR spectra agreed with the indicated structure.

C)

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide, trihydrochloride

51.6 g of 1,4,7,10-tetraazacyclododecane (0.3 mol) and 258.75 g of 2-chloro-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide (0.9 mol), obtained according to the process described in example 7B, were reacted at 70° C. in DMF for 24 h.

After evaporation of the solvent under vacuum, the residue was taken up in water and adsorbed on an ion exchange resin IR 120, from which it was eluted by means of 5N ammonium hydroxide.

The ammonia solution was evaporated to dryness and the residue was transformed into the corresponding trihydrochloride, as described in example 1.

63.84 g of 2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide, trihydrochloride (0.120 mol) were obtained.

Yield: 40.0%; m.p.: 125° C. (dec.).

Elemental analysis: % calc: C 47.33; H 7.56; Cl 19.96; N 13.14. % found: C 47.41; H 7.68; Cl 19.85; N 13.08.

HPLC: 97.6%.

Analogously, the following compounds were obtained:

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-propionamide.

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-N-(2-hydroxyethyl)-propionamide.

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-N-(2,3-dihydroxypropyl)-propionamide.

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-N,N-di(2-hydroxyethyl)-propionamide.

EXAMPLE 8

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl-]-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide.

A mixture of 16 g of 2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide (0.038 mol), obtained according to the process described in example 7, and of 21.13 g of bromoacetic acid (0.152 mol) in 100 ml of water was reacted by the same process as described in example 3, to give the desired compound.

12.4 g of 2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide (0.0207 mol) were obtained.

Yield: 54.4%; m.p.: 137° C. (dec.).

Titre: NaOH) 98.8%.

Elemental analysis: % calc.: C 53.98; H 7.72; N 11.66. % found: C 53.91; H 7.85; N 11.59.

Analogously, the following compounds were obtained:

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-propionamide.

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-N-(2-hydroxyethyl)-propionamide.

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-N-(2,3-dihydroxypropyl)-propionamide.

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-N,N-di(2-hydroxyethyl)-propionamide.

EXAMPLE 9

$Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide

To a suspension of 8 g of 2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide (0.013 mol), obtained according to the process described in example 8, in 30 ml of water 1.33 g of Gd_2O_3 (0.0065 mol) were added and the mixture was reacted at 50° C. according to the procedure of example 5.

The resulting solution was evaporated to dryness to give the desired product.

9 g of $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide (0.012 mol) were obtained.

Yield: 92.3%.

Elemental analysis: % calc.: C 42.95; H 5.74; N 9.28. % found: C 42.87; H 5.80; N 9.23.

HPLC: 97.5%.

EXAMPLE 10

$Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxy-N-(1,3-dihydroxyisopropyl)-propionamide

9 g of $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide (0.012 mol), obtained according to the process described in example 9, were dissolved in 60 ml of water. After addition of 15 g of 5% palladium on charcoal, the solution was hydrogenated according to the procedure of example 6 to give the desired compound.

6.22 g of $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxy-N-(1,3-dihydroxyisopropyl)-propionamide (0.0096 mol) were obtained.

Yield: 80%.

Elemental analysis: % calc: C 36.13; H 5.61; N 10.53. % found: C 36.06; H 5.64; N 10.48.

Analogously, the following compounds were obtained:

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-hydroxy-propionamide

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-hydroxy-N-(2-hydroxyethyl)-propionamide.

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-hydroxy-N-(2,3-dihydroxypropyl)-propionamide.

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-hydroxy-N,N-di-(2-hydroxymethyl)-propionamide.

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxy-propionamide.

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxy-N-(2-hydroxyethyl)-propionamide.

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxy-N-(2,3-dihydroxypropyl)-propionamide

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxy-N,N-di(2-hydroxyethyl)-propionamide.

EXAMPLE 11

Determination of the Relaxivity of the Compounds of the Present Invention

Operative Conditions

Operative conditions	
A) Apparatus:	MINISPEC PC 120 (BRUKER)
B) Observation frequency:	20 MHz (proton)
C) Temperature:	39° C., with pre-thermostatization of the NMR test tube for 10 min at the operative temperature
D) Concentrations:	in the range from 0 to 5 mM with the following specific measuring points: 0/0.1/0.2/0.5/1.0/2.0/5.0 mM
E) Solvent:	0.154 M NaOH (0.9%), water
F) pH:	7.3, to be checked again potentiometrically before the relaxation measurement.

Longitudinal relaxivity (R_1) measurements were calculated using the "Inversion Recovery" sequence with an 8 point minimum and a 3 parameter fit, according to the program provided for the MINISPEC 120 BRUKER instrument, by which measurements were taken.

Transverse relaxivity (R_2) measurements were calculated using the sequence of Carr, Purcell, Meiboom and Gill, according to the program provided for the MINISPEC 120 BRUKER instrument, by means of which measurements were taken, adjusting the apparatus in such a way as to observe the decay of the signal to about ½ of the starting value, with a score number higher or equal to 10 and a 2 parameter fit.

In table I, R_1 and R_2 values calculated for compounds A and B in comparison with Gd/DTPA neutralized with N-methylglucamine, are reported as non-limiting examples.

TABLE I

	A $(mM.s)^{-1}$	B $(mM.s)^{-1}$	Gd/DTPA* $(mM.s)^{-1}$
R_1	4.15 ± 0.01	3.72 ± 0.01	4.08 ± 0.01
R_2	5.67 ± 0.02	5.06 ± 0.01	5.15 ± 0.02

A = $GD^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid, neutralized with N-methylglucamine.
B = $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid, neutralized with N-methylglucamine.
*neutralized with N-methylglucamine; R_1 and R_2 values were determined in aqueous solvent.

EXAMPLE 12

Preparation of liposomes incorporating the $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid complex, neutralized with N-methylglucamine

An anhydrous lipidic mixture was prepared, having the following composition: egg phosphatidylcholine 75 mol % and cholesterol 25 mol % using the REV method (F. Szoka et al., (1978), Proc. Natl. Acad. Sci. U.S.A. 75,4194).

400 mg of said mixture were dissolved in 35 ml of chloroform to which 10 ml of a 0.05M solution of N-methyl-D-glucamine salt of $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic complex acid were added dropwise under sonication. When the addition was over, sonication was continued for 5 min, then the crude compound was heated to 50° C. and the solvent was evaporated under vacuum. The resulting gelly residue was suspended in a 1% NaCl solution and freed from unincorporated chelate by means of five consecutive centrifugations and resuspension steps (26.000 g/10 min).

EXAMPLE 13

Determination of LD_{50} in the Mouse by Intravenous Administration of the Compounds of the Present Invention

In table II are reported, as non-limiting examples, the LD_{50} values for compounds A and B of the present invention, in comparison with $GdCl_3$ and with Gd/DTPA neutralized with N-methylglucamine.

TABLE II

	LD_{50} in the mouse* in mmol/kg - intravenous
$GdCl_3$	0.28 (0.24–0.32)
Gd/DTPA**	4.8 (4.47–5.16)
A	8.8 (7.79–9.94)
B	13.1 (12.2–14.1)

*male and female mice were used, Strain: Crl:CD1(ICR)BR
**N-methylglucamine salt.
A = $GD^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid, neutralized with N-methylglucamine.
B = $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid, neutralized with N-methylglucamine.

Table II shows that, in this pharmacological test, gadolinium complexes with the macrocyclic chelating agents of the invention have substantially decreased toxicities with respect to both $GdCl_3$ and Gd/DTPA.

EXAMPLE 14

Preparation of a solution of D(−)-N-methylglucamine salt of $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid complex

436.8 g (0.500 mol) of the compound obtained according to the procedure described in example 5 were dissolved in 300 ml of pro iniectione (p.i.) water. The solution volume was taken to 500 ml by addition of water p.i., then the solution was filtered, put in vials and sterilized.

EXAMPLE 15

Preparation of a solution of D(−)-N-methylglucamine salt of $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid complex

398.8 g (0.500 mol) of the compound obtained according to the procedure described in example 6, were dissolved in 300 ml of water p.i. The solution volume was taken to 500 ml by addition of water p.i., then the solution was filtered, put in vials and sterilized.

EXAMPLE 16

Preparation of a solution of D(−)-N-methylglucamine salt of $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid complex

218.4 g (0.250 mol) of the salt cited in example 14 were dissolved in 260 ml of water p.i., 0.6 g of ascorbic acid were added and the solution was diluted to 500 ml with water p.i. The solution was sterilized by filtration and put in vials.

EXAMPLE 17

Preparation of a solution of the D(−)-N-methylglucamine salt of $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid complex

218.4 g (0.250 mol) of the salt cited in example 14 were dissolved in 200 ml of water p.i., 0.45 g of tromethamine hydrochloride were added and the solution was diluted to 500 ml with water p.i. The solution was filtered, put in vials and sterilized.

EXAMPLE 18

Preparation of a solution of the D(−)-N-methylglucamine salt of $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid complex

199.4 g (0.250 mol) of the salt cited in example 15 were dissolved in 200 ml of water p.i., 0.6 g of ascorbic acid were added and the solution was diluted to 500 ml with water p.i. The solution was sterilized by filtration and put into vials.

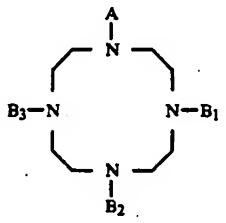
EXAMPLE 19

Preparation of a solution of the D(−)-N-methylglucamine salt of $Gd^{(3+)}$/2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid complex

199.4 g (0.250 mol) of the salt cited in example 15 were dissolved in 200 ml of water p.i., 0.45 g of tromethamine hydrochloride were added and the solution was diluted to 500 ml with water p.i. The solution was filtered, put into vials and sterilized.

We claim:

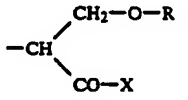
1. A 1, 4, 7, 10-tetraazacyclododecane compound of formula I



I

wherein

A is a group of formula

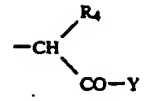


in which

R is H or a C_1–C_5 straight or branched alkyl group, or a benzyl group which is unsubstituted or mono- or poly-substituted on the aromatic ring by halogen, hydroxy, carboxy, carbamoyl, alkoxycarbonyl, sulphamoyl, lower alkyl, lower hydroxyalkyl, amino, acylamino, acyl, hydroxyacyl groups, or a group of formula $H(OCH_2CH_2)_{1-4}$—, $Me(OCH_2CH_2)_{1-4}$—,

X is a O—R_1 group in which R_1 is H or a C_1–C_5 alkyl, hydroxyalkyl alkoxyalkyl, alkoxyhydroxyalkyl group, or a polyoxaalkyl group having 1 to 15 oxygen atoms and 3 to 45 carbon atoms, or X is a —NR_2R_3 group in which R_2 and R_3, are the same or different, and are H, C_1–C_6 alkyl, hydroxyalkyl, alkoxyalkyl or alkoxyhydroxyalkyl groups having up to 5 hydroxy groups and

B_1, B_2 and B_3, are the same or different, and have the same meaning as A or they are H or a group of formula



in which

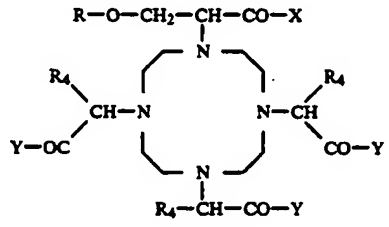
R_4 is H or a C_1–C_5 straight or branched alkyl group,

Y is a O—R_5 group in which R_5 is H or a C_1–C_5 alkyl, hydroxyalkyl, alkoxyalkyl, alkoxyhydroxyalkyl group, or a polyoxyalkyl group having 1 to 15 oxygen atoms and 3 to 45 carbon atoms, or Y is a —NR_6R_7 group in which R_6 and R_7, are the same or different, and are H or C_1–C_6 alkyl, hydroxyalkyl, alkoxyalkyl or alkoxyhydroxyalkyl groups having up to 5 hydroxy groups,

and a salt of said compound of formula I with an organic base which is a member selected from the group consisting of primary, secondary, tertiary amines or with a basic aminoacid or with an inorganic base having a cation which is sodium, potassium or lithium,

and a chelate of said compound of formula I or of a salt thereof with a di- or trivalent ion of a metal element having atomic number ranging from 20 to 31,39,42 to 44,49, 57 to 83, wherein said chelate is neutral or acidic or salified with an organic base which is a member selected from the group consisting of primary, secondary, tertiary amines or with a basic aminoacid or with an inorganic base having a cation which is sodium, potassium or lithium.

2. 1,4,7,10-Tetraazacyclododecane derivatives of general formula II

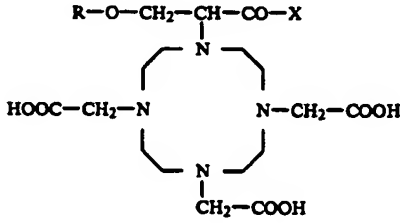


II

wherein

R,R_4,X and Y have the meanings defined in claim 1, and the chelates thereof with appropriate bi- or trivalent ions of metal elements having atomic numbers from 20 to 31, 39, 42, 43, 44, 49 or from 57 to 83.

3. 1,4,7,10-Tetraazacyclododecane derivatives of general formula III

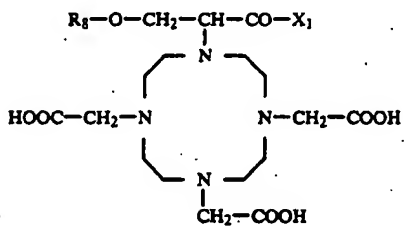


III

wherein R and X have the meanings defined in claim 1, and the chelates thereof with appropriate bi- or trivalent

ions of metal elements having atomic numbers from 20 to 31, 39, 42, 43, 44, 49 or from 57 to 83.

4. Serine derivatives of general formula IV



IV

wherein R_8 is H or benzyl, X_1 is OH, $-NH_2$, $-NHCH_2CH_2OH$, $-NHCH(CH_2OH)_2$, $-NHCH_2CH(OH)CH_2OH$, $-N(CH_2CH_2OH)_2$, $-NH-CH_2-CH(OH)-CH_2OCH_3$ or $-NH-CH_2-CH(OH)-CH(OH)-CH_2OH$, and the chelates thereof with appropriate bi- or trivalent ions of metal elements having atomic numbers from 20 to 31, 38, 42, 43, 44, 49, or from 57 to 83.

5. A chelate of a compound as claimed in claim 1, in which the chelate metal ion if $Fe^{(2+)}$, $Fe^{(3+)}$, $Cu^{(2+)}$, $Gd^{(3+)}$, $Eu^{(3+)}$, $Dy^{(3+)}$ or $Mn^{(2+)}$.

6. Chelates as claimed in claim 2 with the ions of the following radioisotopes: ^{51}Cr, ^{68}Ga, ^{111}In, ^{99m}Tc, ^{140}La, ^{168}Yb.

7. A compound as claimed in claim 1, selected from the group consisting of:

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-propionic acid,

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-hydroxypropionic acid,

2-[1,4,7,10-tetraaza-4-(1-carboxy-2-benzyloxy-ethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid,

2-[1,4,7,10-tetraaza-4-(1-carboxy-2-hydroxy-ethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid,

2-[1,4,7,10-tetraaza-7-(1-carboxy-2-benzyloxy-ethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid,

2-[1,4,7,10-tetraaza-7-(1-carboxy-2-hydroxy-ethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid,

2-[1,4,7,10-tetraaza-4,7-di(1-carboxy-2-benzyloxy-ethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid,

2-[1,4,7,10-tetraaza-4,7-di(1-carboxy-2-hydroxy-ethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid,

2-[1,4,7,10-tetraaza-4-(1-carboxy-2-benzyloxy-ethyl)-7,10-di(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid,

2-[1,4,7,10-tetraaza-4-(1-carboxy-2-hydroxy-ethyl)-7,10-di(carboxymethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid,

2-[1,4,7,10-tetraaza-7-(1-carboxy-2-benzyloxy-ethyl)-4,10-di(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid,

2-[1,4,7,10-tetraaza-7-(1-carboxy-2-hydroxy-ethyl)-4,10-di(carboxymethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid,

2-[1,4,7,10-tetraaza-4,7-di(1-carboxy-2-benzyloxy-ethyl)-10-carboxymethyl-cyclododecane-1-yl]-3-benzyloxypropionic acid,

2-[1,4,7,10-tetraaza-4,7-di(1-carboxy-2-hydroxy-ethyl)-10-carboxymethyl-cyclododecane-1-yl]-3-hydroxy-propionic acid,

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxypropionic acid,

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxypropionic acid,

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-propionamide,

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-hydroxy-propionamide,

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-N-(2-hydroxyethyl)-propionamide,

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-hydroxy-N-(2-hydroxyethyl)-propionamide,

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide,

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-hydroxy-N-(1,3-dihydroxyisopropyl)-propionamide,

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-N-(2,3-dihydroxypropyl)-propionamide,

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-hydroxy-N-(2,3-dihydroxypropyl)-propionamide,

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-benzyloxy-N,N-di(2-hydroxyethyl)-propionamide,

2-(1,4,7,10-tetraazacyclododecane-1-yl)-3-hydroxy-N,N-di-(2-hydroxyethyl)-propionamide,

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-propionamide,

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxy-propionamide,

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-N-(2-hydroxyethyl)-propionamide,

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxy-N-(2-hydroxyethyl)-propionamide,

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-N-(1,3-dihydroxyisopropyl)-propionamide,

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxy-N-(1,3-dihydroxyisopropyl)-propionamide,

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-N-(2,3-dihydroxypropyl)-propionamide,

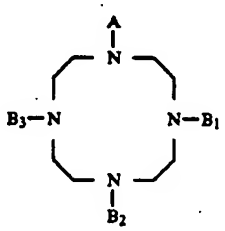
2-[1,4,7,10-tetraza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxy-N-(2,3-dihydroxypropyl)-propionamide,

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-N,N-di(2-hydroxyethyl)-propionamide,

2-[1,4,7,10-tetraaza-4,7,10-tri(carboxymethyl)-cyclododecane-1-yl]-3-hydroxy-N,N-di(2-hydroxyethyl)-propionamide,

the respective chelate complexes with $Fe^{(3+)}$, $Cu^{(2+)}$, $Mn^{(2+)}$, $Gd^{(3+)}$, $Dy^{(3+)}$, $In^{(3+)}$, $La^{(3+)}$, $Yb^{(3+)}$ and the corresponding salts with D(−)-N-methylglucamine.

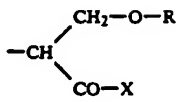
8. A method for the preparation of a metal chelate of a 1,4,7,10-tetraazacyclododecane compound of formula I



I

wherein

A is a group of formula

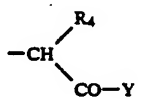


in which

R is H or a C_1–C_5 straight or branched alkyl group, or a benzyl group which is unsubstituted or mono- or poly-substituted on the aromatic ring by halogen, hydroxy, carboxy, carbamoyl, alkoxycarbonyl, sulphamoyl, lower alkyl, lower hydroxyalkyl, amino, acylamino, acyl, hydroxyacyl groups, or a group of formula $H(OCH_2CH_2)_{1-4}$—, $Me(OCH_2CH_2)_{1-4}$— or $Et(OCH_2CH_2)_{1-4}$—,

X is a O—R_1 group in which R_1 is H or a C_1–C_5 alkyl, hydroxyalkyl, alkoxyalkyl, alkoxyhydroxyalkyl group, or a polyoxaalkyl group having 1 to 15 oxygen atoms and 3 to 45 carbon atoms, or X is a —NR_2R_3 group in which R_2 and R_3, are the same or different, and are C_1–C_6 alkyl, hydroxyalkyl, alkoxyalkyl or alkoxyhydroxyalkyl groups having up to 5 hydroxy groups and

B_1, B_2 and B_3, are the same or different, and have the same meaning as A or they are H or a group of formula



in which

R_4 is H or a C_1–C_5 straight or branched alkyl group, Y is a O—R_5 group in which R_5 is H or a C_1–C_5 alkyl, hydroxyalkyl, alkoxyalkyl, alkoxyhydroxyalkyl group, or a polyoxaalkyl group having 1 to 15 oxygen atoms and 3 to 45 carbon atoms, or Y is a —NR_6R_7 group in which R_6 and R_7 are the same or different, and are H or C_1–C_6 alkyl, hydroxyalkyl, alkoxyalkyl or alkoxyhydroxyalkyl groups having up to 5 hydroxy groups, which consists in reacting a salt or an oxide of a metal selected from the metals having atomic numbers from 20 to 31, 39,42,43,44,49 or from 57 to 83 with said compound of formula I or a salt thereof in the presence of an acid or a base in an amount necessary for neutralization.

9. A compound according to claim 1 which is the D(−)-N-methylglucamine salt of the chelate complex of Gd(3+) with 2-[1,4,7,10-tetraaza-4,7-di(1-carboxy-2-benzyloxy-ethyl)-10-carboxymethyl-cyclododecane-1-yl]-3-benzyloxypropionic acid.

10. A compound according to claim 1 which is the Gd (3+)complex with 2-[1,4,7,10-tetraaza-4,7,10-tri(-carboxymethyl)-cyclododecane-1-yl]-3-benzyloxy-[N-methyl-N(D-1-deoxyglucitol)]-propionamide.

11. A compound according to claim 1 which is the Gd(3+)complex with 2-[1,4,7,10-tetraaza-4,7,10-tri(-carboxymethyl)-cyclododecane-1-yl]-3-(L)-benzyloxy-N-methyl-N(D-1-deoxyglucitol)]-propionamide.

12. A compound according to claim 1 which is Gd(3+)complex with 2-[1,4,7,10-tetraaza-4,7,10-tri(-carboxymethyl)-cyclododecane-1-yl]3-hydroxy-[N-methyl-N-(D)-1-deoxy-glucitol)]-propionamide.

13. A compound according to claim 1 which is the Gd(3+)complex with 2-[1,4,7,10-tetraaza-4,7,10-tri(-carboxymethyl)-cyclododecane-1-yl]3-(L)-hydroxy-[N-methyl-N-(D-1-deoxyglucitol)]-propionamide.

14. The compound according to claim 1 wherein said organic base is ethanolamine, diethanolamine, morpholine, glucamine, N,N-dimethylglucamine or N-methylglucamine.

15. The compound according to claim 1 wherein said basic aminoacid is lysine, arginine or omithine.

16. The compound according to claim 1 wherein said inorganic base is sodium hydroxide, potassium hydroxide or lithium hydroxide.

17. The method according to claim 8 wherein said acid necessary for the neutralization is an inorganic or organic acid, said inorganic acid having an anion which is a member selected from the group consisting of chloride, bromide, iodide or sulfate, said organic acid being acetic, succinic, citric, fumaric or maleic.

18. The method according to claim 8 wherein said base necessary for neutralization is an inorganic or organic base, said inorganic base having a cation which is sodium, potassium or lithium, said organic base being a primary, secondary or tertiary amine or a basic aminoacid.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,132,409
DATED : July 21, 1992
INVENTOR(S) : Ernst Felder, Carlo Musu, Luciano Fumagalli, Fulvio Uggeri

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

On the title page: Item [30] FOREIGN PRIORITY DATA should read --23217 A/87--.

Signed and Sealed this

Tenth Day of January, 1995

Attest:

BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks

US006466814B1

(12) **United States Patent**
Ardenkjaer-Larsen et al.

(10) **Patent No.: US 6,466,814 B1**
(45) **Date of Patent: *Oct. 15, 2002**

Please SCAN

(54) **METHOD OF MAGNETIC RESONANCE INVESTIGATION**

(75) Inventors: **Jan Henrik Ardenkjaer-Larsen; Oskar Axelsson; Klaes Golman; Lars-Goran Wistrand; Georg Hansson**, all of Malmo (SE); **Ib Leunbach**, Dragor (DK); **Stefan Petersson**, Malmo (SE)

(73) Assignee: **Amersham Health AS**, Oslo (NO)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 34 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/609,153**

(22) Filed: **Jun. 30, 2000**

Related U.S. Application Data

(63) Continuation of application No. PCT/GB98/03904, filed on Dec. 23, 1998.
(60) Provisional application No. 60/076,924, filed on Mar. 5, 1998.

(30) **Foreign Application Priority Data**

Jan. 5, 1998 (GB) 9800158
Jun. 25, 1998 (GB) 9813795

(51) **Int. Cl.**[7] **A61B 5/05**
(52) **U.S. Cl.** **600/420**; 600/419; 424/9.3; 324/307; 324/309
(58) **Field of Search** 600/410, 420, 600/419; 324/307, 309; 424/9.3

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,617,859 A 4/1997 Souza et al.
6,278,893 B1 * 8/2001 Ardenkjaer-Larson et al. ... 600/420

FOREIGN PATENT DOCUMENTS

WO 0 355 884 A 2/1990
WO WO 97 37239 A 10/1997
WO WO 98/01766 A 1/1998
WO WO 98 30918 A 7/1998

OTHER PUBLICATIONS

Chapellier M. et al., "First observation of a dynamic polarization of monolayers of He/sup 3/ adsorbed on fluorocarbon microspheres", Proceedings of the 17th International Conference on Low Temperature Physics, Lt–17, Karlsruhe, West Germany, Aug. 15–22, 1984, XP002098873.

Langer S.A. et al., "A proposed method for polarizing liquid /sup 3/He", Journal of Low Temperature Physics, Nov. 1984, XP002098874.

Pietrass T. et al., "Optically polarized /sup 129/Xe in NMR spectroscopy", Advanced Materials, Oct. 1995, VCH Verlagsgesellschaft, Germany, XP002098966.

Disclosed anonymously, "The Use of Dynamically Polarized Contrast Agents", 4/93, XP002070308.

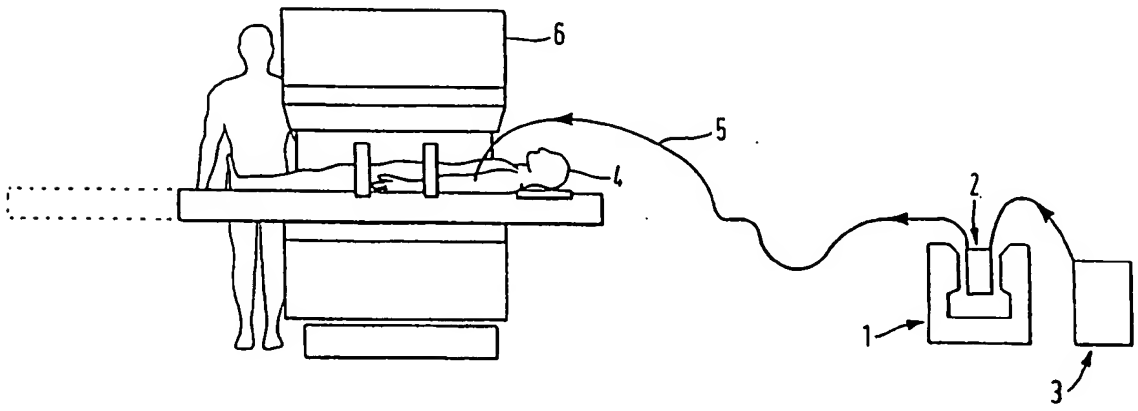
* cited by examiner

Primary Examiner—Ruth S. Smith
(74) *Attorney, Agent, or Firm*—Bacon & Thomas

(57) **ABSTRACT**

The present invention provides a method of magnetic resonance investigation of a sample, preferably of a human or non-human animal body. The method comprises the step of ex vivo polarization of a high T_1 agent. The polarizing agent is optionally separated from the high T_1 agent before the high T_1 agent is administered to the sample.

34 Claims, 5 Drawing Sheets



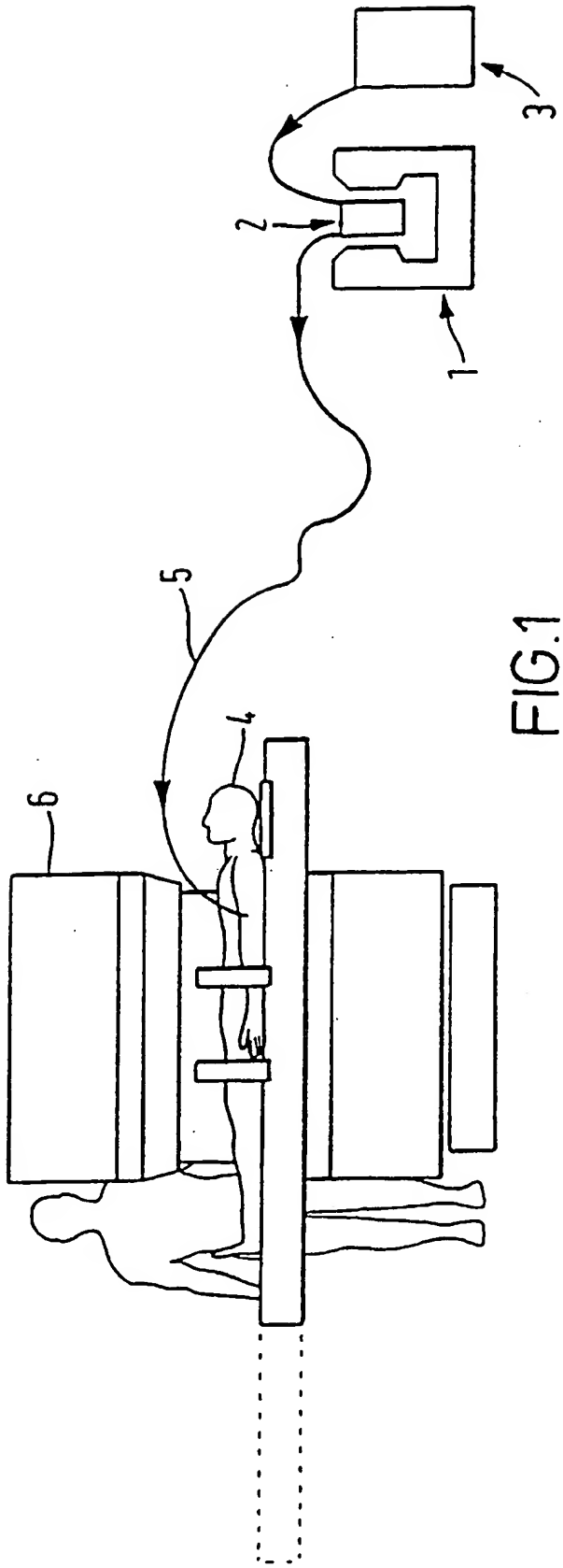


FIG.1

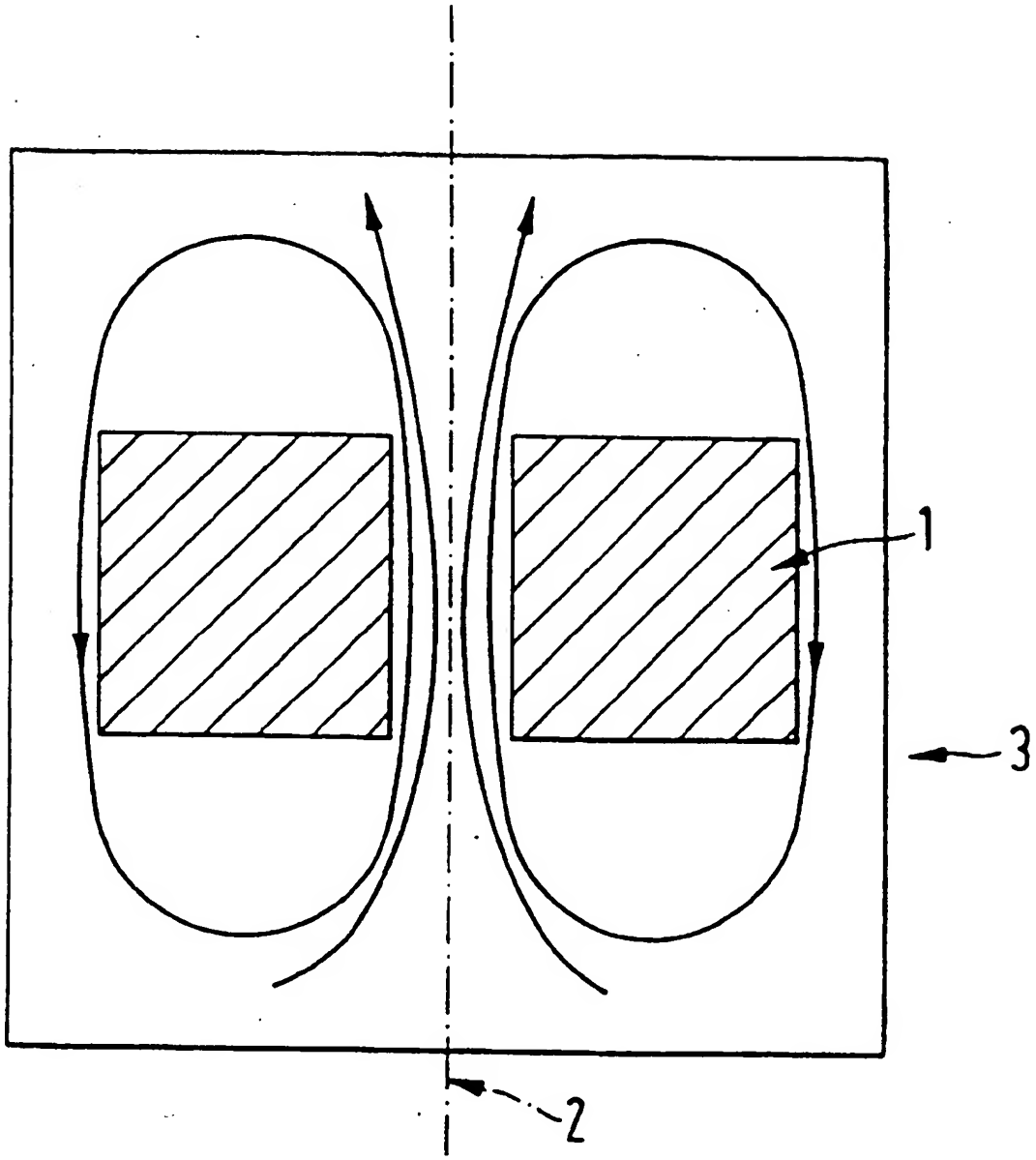


FIG. 2

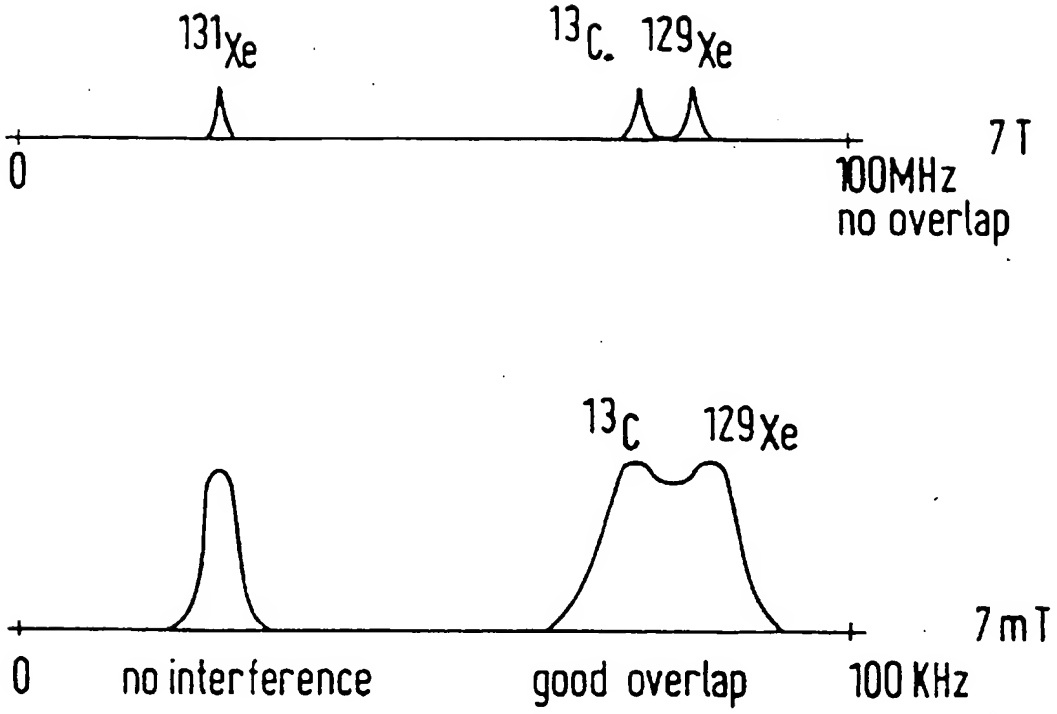

131Xe
13C. 129Xe
0
100MHz
no overlap
7 T
13C 129Xe
0
no interference
good overlap
100 KHz
7 mT

FIG.3

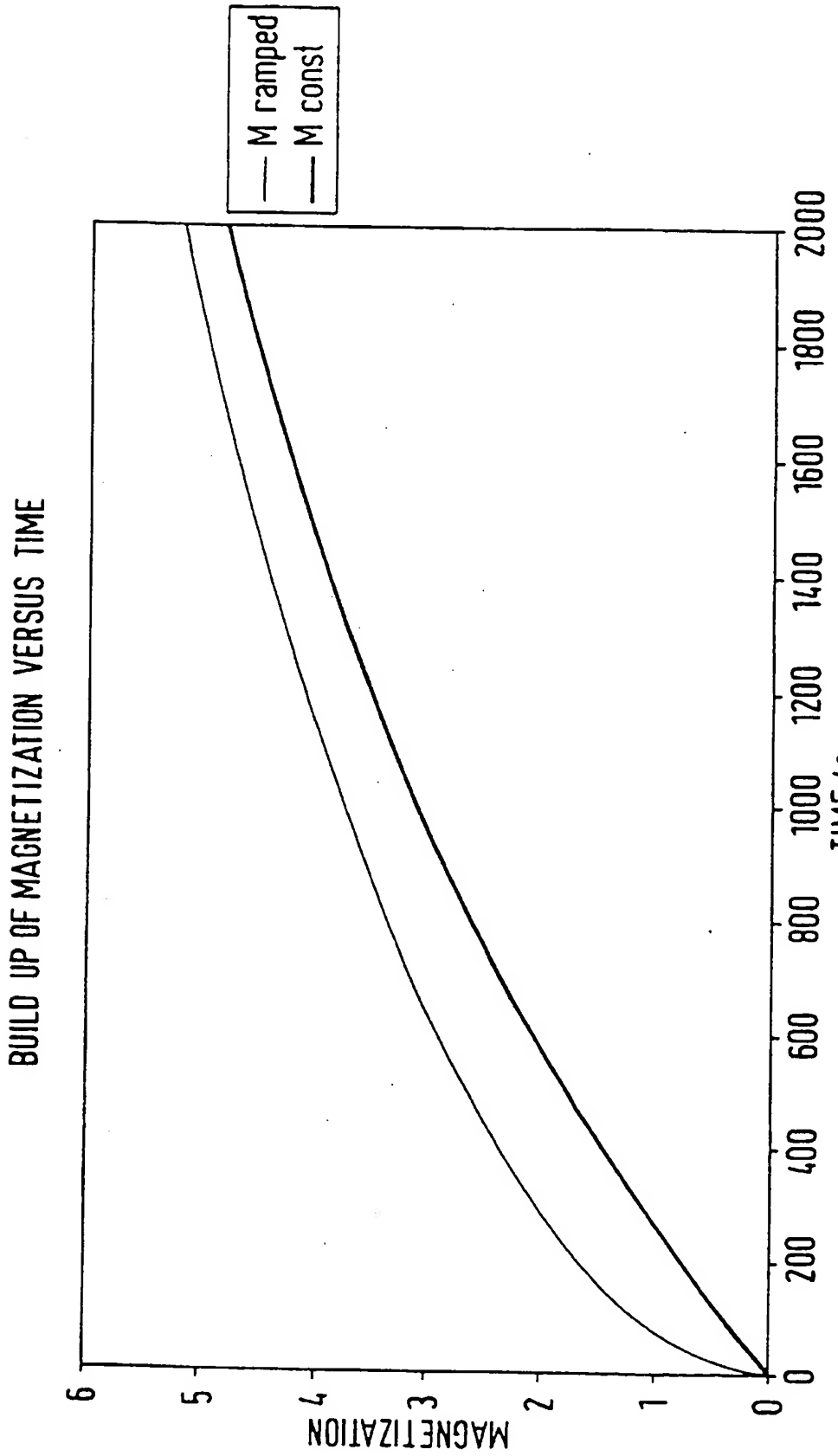
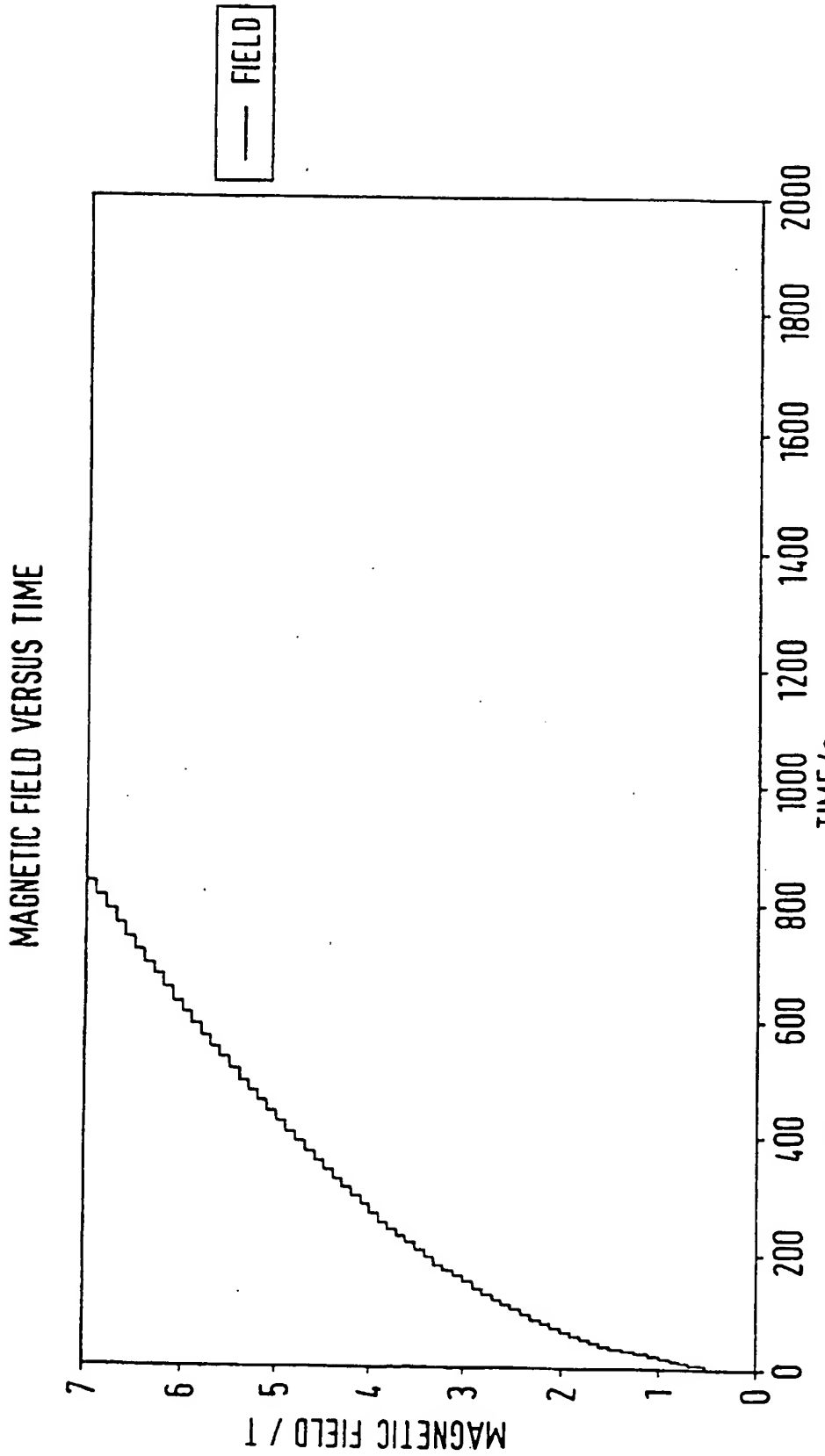

M ramped
M const
BUILD UP OF MAGNETIZATION VERSUS TIME
MAGNETIZATION
0
1
2
3
4
5
6
0
200
400
600
800
1000
1200
1400
1600
1800
2000
TIME/s

FIG.4


FIELD
MAGNETIC FIELD VERSUS TIME
MAGNETIC FIELD / T
TIME/s
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1
2
3
4
5
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7
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200
400
600
800
1000
1200
1400
1600
1800
2000

FIG.5

METHOD OF MAGNETIC RESONANCE INVESTIGATION

This application is a continuation of international application number PCT/GB98/03904, filed Dec. 23, 1998 (of which the entire disclosure of the pending, prior application is hereby incorporated by reference), which claims benefit of a continuation-in-part of U.S. provisional application No. 60/076,924, filed Mar. 5, 1998.

This invention relates to a method of magnetic resonance imaging (MRI).

Magnetic resonance imaging (MRI) is a diagnostic technique that has become particularly attractive to physicians as it is non-invasive and does not involve exposing the patient under study to-potentially harmful radiation such as X-rays.

In order to achieve effective contrast between MR images of the different tissue types in a subject, it has long been known to administer to the subject MR contrast agents (e.g. paramagnetic metal species) which effect relaxation times of the MR imaging nuclei in the zones in which they are administered or at which they aggregate. Contrast enhancement has also been achieved by utilising the "Overhauser effect" in which an esr transition in an administered paramagnetic species (hereinafter an OMRI contrast agent) is coupled to the nuclear spin system of the imaging nuclei. The Overhauser effect (also known as dynamic nuclear polarisation) can significantly increase the population difference between excited and ground nuclear spin states of selected nuclei and thereby amplify the MR signal intensity by a factor of a hundred or more allowing OMRI images to be generated rapidly and with relatively low primary magnetic fields. Most of the OMRI contrast agents disclosed to date are radicals which are used to effect polarisation of imaging nuclei in vivo.

EP-A-0355884 (to Hafslund Nycomed Innovation AB) discloses a method of and apparatus for performing electron spin resonance enhanced magnetic resonance imaging (ESREMRI) at ultra-low fields of up to 20 Gauss. Research Disclosure No. 348, April 1993, 242 (anon) discloses that electron paramagnetic resonance can result in the enhancement of an MR signal.

Techniques are now being developed which involve ex vivo polarisation of agents containing MR imaging nuclei, prior to administration and MR signal measurement. Such techniques may involve the use of polarising agents, for example conventional OMRI contrast agents or hyperpolarised gases to achieve ex vivo polarisation of administrable MR imaging nuclei. By polarising agent is meant any agent suitable for performing ex vivo polarisation of an MR imaging agent.

The ex vivo method has inter alia the advantage that it is possible to avoid administering the whole of, or substantially the whole of, the polarising agent to the sample under investigation, whilst still achieving the desired polarisation. Thus the method is less constrained by physiological factors such as the constraints imposed by the administrability, biodegradability and toxicity of OMRI contrast agents in in vivo techniques.

It has now been found that ex vivo methods of magnetic resonance imaging may be improved by using polarised MR imaging agents comprising nuclei capable of emitting magnetic resonance signals in a uniform magnetic field (eg MR imaging nuclei such as ^{13}C or ^{15}N nuclei) and capable of exhibiting a long T_1 relaxation time, preferably additionally a long T_2 relaxation time. Such agents will be referred to hereinafter as "high T_1 agents". Typically the molecules of a high T_1 agent will contain MR imaging nuclei in an amount greater than the natural abundance of said nuclei in said molecules (i.e. the agent will be enriched with said nuclei).

Thus viewed from one aspect the present invention provides a method of magnetic resonance investigation of a sample, preferably of a human or non-human animal body (eg. a mammalian, reptilian or avian body), said method comprising:

(i) producing a hyperpolarised solution of a high T_1 agent by dissolving in a physiologically tolerable solvent a hyperpolarised solid sample of said high T_1 agent;

(ii) where the hyperpolarisation of the solid sample of said high T_1 agent in step (i) is effected by means of a polarising agent, optionally separating the whole, substantially the whole, or a portion of said polarising agent from said high T_1 agent;

(iii) administering said hyperpolarised solution to said sample;

(iv) exposing said sample to radiation of a frequency selected to excite nuclear spin transitions in selected nuclei eg the MR imaging nuclei of the high T_1 agent;

(v) detecting magnetic resonance signals from said sample; and

(vi) optionally, generating an image, dynamic flow data, diffusion data, perfusion data, physiological data (eg. pH, PO_2, pCO_2, temperature or ionic concentrations) or metabolic data from said detected signals,

wherein said high T_1 agent in said hyperpolarised solution has a T_1 value (at a field strength in the range 0.01–5 T and a temperature in the range 20–40° C.) of at least 5 seconds and furthermore wherein said high T_1 agent is ^{13}C enriched at one or more carbonyl or quaternary carbon positions.

Thus the invention involves the sequential steps of producing a hyperpolarised solution from a hyperpolarised solid sample of a high T_1 agent comprising nuclei capable of exhibiting a long T_1 relaxation time, administration of the hyperpolarised solution of the high T_1 agent (preferably in the absence of a portion of, more preferably substantially the whole of, any polarising agent), and conventional in vivo MR signal generation and measurement. The MR signals obtained in this way may be conveniently converted by conventional manipulations into 2-, 3- or 4-dimensional data including flow, diffusion, physiological or metabolic data.

By "hyperpolarised" we mean polarised to a level over that found at room temperature and 1 T, preferably polarised to a polarisation degree in excess of 0.1%, more preferably 1%, even more preferably 10%.

Polarization is given by the equation

$$P = \left| \frac{N\alpha - N\beta}{N\alpha + N\beta} \right|$$

which at equilibrium is equal to

$$\frac{1 - \exp(-\gamma\hbar B_o / kT)}{1 + \exp(-\gamma\hbar B_o / kT)}$$

where

$N\alpha$ is the number of spins in nuclear spin state α (e.g. +½);

N_β is the number of spins in nuclear spin state β (e.g. -½);

γ is the magnetogyric ratio for the isotopic nucleus in question, e.g. ^{13}C);

$\hbar$ is Planck's constant divided by 2n;

B_G is the magnetic field;

k is Boltzmann's constant; and

T is temperature in kelvin.

Thus P has a maximum value of 1 (100% polarization) and a minimum value of 0 (0% polarization).

By "physiologically tolerable solvent" we mean any solvent, solvent mixture or solution that is tolerated by the human or non-human animal body, e.g. water, aqueous solutions such as saline, perfluorocarbons, etc.

One embodiment of the invention provides a method as described above wherein the hyperpolarised solid sample of said high T_1 agent retains its polarisation when transported in a magnetic field and at low temperature; in this way the agent can be hyperpolarised at a site remote from its end use and transported to its place of use in a magnetic field and at a low temperature and there dissolved and administered.

In the embodiment referred to above, the magnetic field is preferably greater than 10 mT, more preferably greater than 0.1 T, even more preferably greater than 0.5 T, yet more preferably greater than 1 T. By "low temperature" we preferably mean lower than 80 K, more preferably lower than 4.2 K, most preferably lower than 1 K.

A further embodiment of the invention provides a method as described above wherein the hyperpolarised solution thus formed retains its polarisation when transported in a magnetic field. In this latest embodiment, the magnetic field is preferably greater than 10 mT, more preferably greater than 0.1 T, even more preferably greater than 0.5 T, yet more preferably greater than 1 T.

A yet further embodiment of the invention provides a method as described above wherein a magnetic field is present during the dissolution stage. In this latest embodiment, the magnetic field is preferably greater than 10 mT, more preferably greater than 0.1 T, even more preferably greater than 0.5 T, yet more preferably greater than 1 T.

Suitable high T_1 agents may contain nuclei such as protons. However other non-zero nuclear spin nuclei may be useful (eg ^{19}F, ^{3}Li, ^{13}C, ^{15}N, ^{29}Si or ^{31}P, as well as ^{1}H), preferably ^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{29}Si and ^{31}P nuclei, with ^{13}C and ^{15}N nuclei being particularly preferred. In this event the MR signals from which the image is generated will be substantially only from the high T_1 agent itself. Nonetheless, where the polarised high T_1 agent is present in high concentration in administrable media, there may be significant enough transfer of magnetisation to the protons to be able to perform ^{1}H-MRI on the protons of the media. Similarly, the polarised high T_1 agent may have a significant enough effect on in vivo protons for conventional ^{1}H-MRI to be carried out on those protons.

Where the MR imaging nuclei is other than a proton (eg ^{13}C or ^{15}N) , there will be essentially no interference from background signals (the natural abundance of ^{13}C and ^{15}N being negligible) and image contrast will be advantageously high. This is especially true where the high T_1 agent itself is enriched above natural abundance. Thus the method according to the invention has the benefit of being able to provide significant spatial weighting to a generated image. In effect, the administration of a polarised high T_1 agent to a selected region of a sample (eg by injection) means that the contrast effect may be localised to that region. The precise effect of course depends on the extent of biodistribution over the period in which the high T_1 agent remains significantly polarised. In general, specific body volumes (i.e. regions of interest such as the vascular system or specific organs such as the brain, kidney, heart or liver) into which the agent is administered may be defined with improved signal to noise (particularly improved contrast to noise) properties of the resulting images in these volumes.

In one embodiment, a "native image" of the sample (e.g. body) (ie. one obtained prior to administration of the high T_1 agent or one obtained for the administered high T_1 agent without prior polarisation as in a conventional MR experiment) may be generated to provide structural (eg. anatomical) information upon which the image obtained in the method according to the invention may be superimposed. A "native image" is generally not available where ^{13}C or ^{15}N is the imaging nucleus because of the low abundance of ^{13}C and ^{15}N in the body. In this case, a proton MR image may be taken to provide the anatomical information upon which the ^{13}C or ^{15}N image may be superimposed.

The high T_1 agent should of course be physiologically tolerable or be capable of being provided in a physiologically tolerable, administrable form. Preferred high T_1 agents are soluble in aqueous media (eg. water) and are of course non-toxic where the intended end use is in vivo.

Conveniently, the high T_1 agent once polarised will remain so for a period sufficiently long to allow the imaging procedure to be carried out in a comfortable time span. Generally sufficient polarisation will be retained by the high T_1 agent in its administrable form (eg. in injection solution) if it has a T_1 value (at a field strength of 0.01–5 T and a temperature in the range 20–40° C.) of at least 5 s, more preferably at least 10 s, especially preferably 30 s or longer, more especially preferably 70 s or more, yet more especially preferably 100 s or more (for example at 37° C. in water at 1 T and a concentration of at least 1 mM). The high T_1 agent may be advantageously an agent with a long T_2 relaxation time.

The long T_1 relaxation time of certain ^{13}C nuclei is particularly advantageous and certain high T_1 agents containing ^{13}C nuclei are therefore preferred for use in the present method. The γ-factor of carbon is about ¼ of the γ-factor for hydrogen resulting in a Larmor frequency of about 10 MHz at 1 T. The rf-absorption and reflections in a patient is consequently and advantageously less than in water (proton) imaging. The signal-to-noise ratio is found to be independent of the MRI field strength when the corresponding frequency is higher than a few MHz. Preferably the polarised high T_1 agent has an effective ^{13}C nuclear polarisation corresponding to the one obtained at thermal equilibrium at 300 K in a field of 0.1 T or more, more preferably 25 T or more, particularly preferably 100 T or more, especially preferably 5000 T or more (for example 50 kT)

When the electron cloud of a given molecule interacts with atoms in surrounding tissue, the shielding of the atom responsible for the the MR signal is changed giving rise to a shift in the MR frequency ("the chemical shift effect"). When the molecule is metabolised, the chemical shift will be changed and high T_1 agents in different chemical surroundings may be visualised separately using pulses sensitive to chemical shift. When the frequency difference between high T_1 molecules in different surroundings is 10 Hz or higher, preferably 20 Hz or higher, most preferably 150 Hz or higher (corresponding to 3.5 ppm or higher at 1 T), the two components may be excited separately and visualised in two images. Standard chemical shift selective excitation pulses may then be utilised. When the frequency separation is less, the two components may not be separated by using frequency selective rf-pulses. The phase difference created during the time delay after the excitation pulse and before the detection of the MR signal may then be used to separate the two components. Phase sensitive imaging pulse sequence methods (Dixon, Radiology, 1984, 153: 189–194 and Sepponen, Mag Res. Imaging, 3, 163–167, 1985) may

be used to generate images visualising different chemcial surroundings or different metabolites. The long T_2 relaxation time which may be a characteristic of a high T_1 agent will under these circumstances make it possible to use long echo times (TE) and still get a high signal-to-noise ratio. Thus an important advantage of the high T_1 agents used in the present method is that they exhibit a chemical shift dependent on the local composition of the body in which they are localized. Preferred high T_1 agents will exhibit at 1 T a chemical shift of more than 2 ppm, preferably more than 10 ppm depending on whether the high T_1 agent is localised inside or outside the vascular system. More preferred high T_1 agents will exhibit a chemical shift of more than 2 ppm, preferably more than 10 ppm, per 2 pH units or per Kelvin or upon being metabolised. High T_1 agents containing polarised ^{13}C nuclei (or ^{15}N nuclei) exhibit large changes in chemical shift in response to physiological changes (eg. pH, pO_2, pCO_2, redox potential, temperature or ionic concentrations of for example Na^+, K', Ca^{2+}) or metabolic activity and therefore may be used to monitor these parameters.

Solid high T_1 agents (e.g. ^{13}C or ^{15}N enriched solids) may exhibit very long T_1 relaxation times and for this reason are especially preferred for use in the present method. The T_1 relaxation time may be several hours in the bulk phase, although this may be reduced by reduction of grain size and/or addition of paramagnetic impurities eg. molecular oxygen. The long relaxation time of solids advantageously allows the procedure to be conveniently carried out with less haste and is particularly advantageous in allowing the polarised solid high T_1 agent to be stored or transported prior to pharmaceutical formulation and administration. In one embodiment, the polarised high T_1 agent may be stored at low temperature and prior to administration, the high T_1 agent may be rapidly warmed to physiological temperatures using conventional techniques such as infrared or microwave radiation or simply by adding hot, sterile administrable media eg saline.

For in vivo use, a polarised solid high T_1 agent is dissolved in administrable media (eg water or saline), administered to a subject and conventional MR imaging performed. Thus solid high T_1 agents are preferably rapidly soluble (eg. water soluble) to assist in formulating administrable media. Preferably the high T_1 agent should dissolve in a physiologically tolerable carrier (eg water or Ringers solution) to a concentration of at least 1 mM at a rate of 1 mM/3 T_1 or more, particularly preferably 1 mM/2 T_1 or more, especially preferably 1 mM/T_1 or more. Where the solid high T_1 agent is frozen, the adminstrable medium may be heated, preferably to an extent such that the temperature of the medium after mixing is close to 37° C.

A polarised high T_1 agent may be administered (either alone or with additional components such as additional high T_1 agents) in liquid form. The retention of polarisation in a liquid medium vis-a-vis a gas medium is significantly greater. Thus while T_1 and T_2 are in general shorter for the liquid, the T_2^* effect due to diffusion is 10^5 times less significant for the liquid. Consequently for gaseous high T_1 agents the imaging sequence used generally has to be FLASH or GRASS while in contrast, more efficient imaging sequences may be used for liquids. For example, liquids generally have slower diffusion which makes it possible to use sequences such as echo planar imaging (EPI). The overall technique will be faster and yield better resolution (voxel size <1 mm) than conventional techniques (voxel size approx. 1–5 mm) at current acquisition times. It will give good images at all fields including in low field (eg. 0.01–0.5 T) machines.

Unless the hyperpolarised agent is stored (and/or transported) at low temperature and in an applied field as described above, since the method of the invention should be carried out within the time that the hyperpolarised solution of the high T_1 agent remains significantly polarised, it is desirable for administration of the polarised high T_1 agent to be effected rapidly and for the MR measurement to follow shortly thereafter. The preferred administration route for the polarised high T_1 agent is parenteral eg by bolus injection, by intravenous, intraarterial or peroral injection. The injection time should be equivalent to 5 T_1 or less, preferably 3 T_1 or less, particularly preferably T_1 or less, especially 0.1 T_1 or less. The lungs may be imaged by spray, eg by aerosol spray.

The high T_1 agent should be preferably enriched with nuclei (eg. ^{15}N and/or ^{13}C nuclei) having a long T_1 relaxation time. Preferred are ^{13}C enriched high T_1 agents having ^{13}C at one particular position (or more than one particular position) in an amount in excess of the natural abundance, i.e. above about 1%. Preferably such a single carbon position will have 5% or more ^{13}C, particularly preferably 10% or more, especially preferably 25% or more, more especially preferably 50% or more, even more preferably in excess of 99% (e.g. 99.9%). The ^{13}C nuclei should preferably amount to >2% of all carbon atoms in the compound. The high T_1 agent is ^{13}C enriched at one or more carbonyl or quaternary carbon postions, given that a ^{13}C nucleus in a carbonyl group or in certain quaternary carbons may have a T_1 relaxation time typically of more than 2 s, preferably more than 5 s, especially preferably more than 30 s. Preferably the ^{13}C enriched compound should be deuterium labelled, especially adjacent the ^{13}C nucleus.

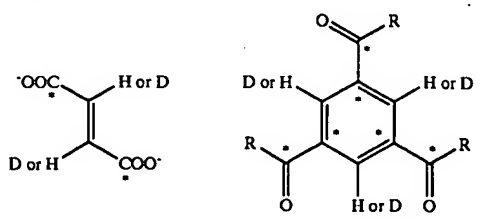
Viewed from a further aspect the present invention provides a composition comprising a hyperpolarised solution of a polarised ^{13}C, ^{15}N, ^{19}F, ^{29}Si, ^{31}P or ^{1}H enriched compound together with one or more physiologically acceptable carriers or excipients.

Viewed from a further aspect the present invention provides a contrast medium comprising a hyperpolarised solution of a polarised high T_1 agent being enriched with ^{13}C, ^{15}N, ^{19}F, ^{29}Si, ^{31}P or ^{1}H nuclei having a T_1 relaxation time of 2 s or more, preferably 10 secs or more, more preferably 30 secs or more, especially preferably 60 secs or more in solution at magnetic fields of 0.005–10 T, preferably 0.01–10 T, together with one or more physiologically acceptable carriers or excipients.

Preferred ^{13}C enriched compounds are those in which the ^{13}C nucleus is surrounded by one or more non-MR active nuclei such as O, S, C or a double bond. Specifically preferred ^{13}C enriched agents are $^{13}CO_3^{2-}$ and $H^{13}CO_3^-$ (sodium salt for injection and calcium or potassium salt for polarisation).

Also preferred are the following types of compound (* denotes ^{13}C enriched positions):

(1) carboxyl compounds comprising 1 to 4 carboxyl groups:



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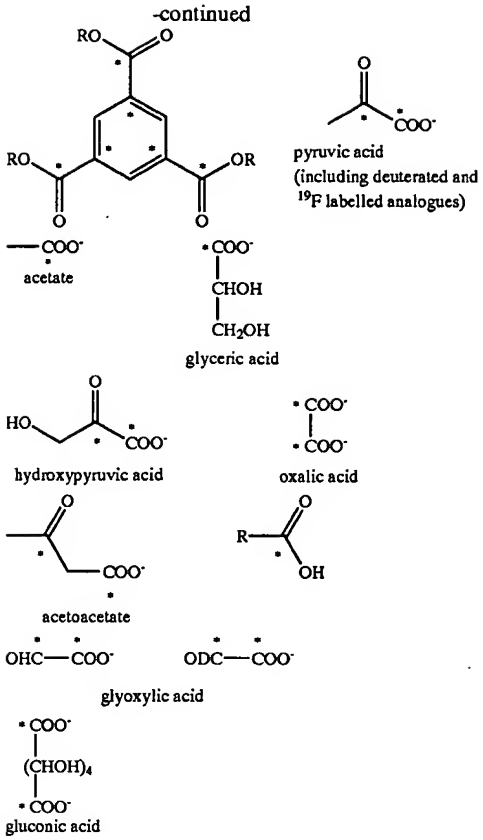
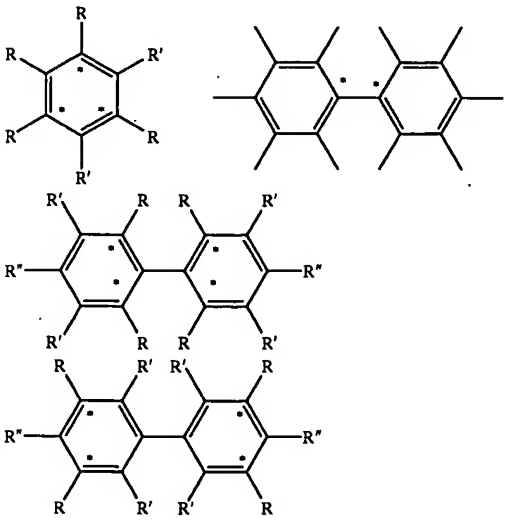


(wherein R represents any straight or branched chain hydrocarbon moiety, preferably a highly substituted carbon atom, especially preferably a quaternary carbon) and esters, isomers, especially stereoisomers and rotamers, thereof;

(2) substituted mono and biaryl compounds:



(wherein each group R or R' is independently a hydrogen atom, an iodine atom, a ^{19}F atom or a hydrophilic moiety M being any of the non-ionizing groups conventionally used to enhance water solubility within the field of triiodophenyl X-ray contrast agents including for example a straight chain or branched C_{1-10}-alkyl group, preferably a C_{1-5} group, optionally with one or more CH_2 or CH moieties replaced by oxygen or nitrogen atoms and optionally substituted by one or more groups selected from oxo, hydroxy, amino, carboxyl derivative, and oxo substituted sulphur and phosphorus atoms).

Particular examples of group M include polyhydroxyalkyl, hydroxyalkoxyalkyl and hydroxypolyalkoxyalkyl and such groups attached to the phenyl group via an amide linkage such as hydroxyalkylaminocarbonyl, N-alkyl-hydroxyalkylaminocarbonyl and bis-hydroxyalkylaminocarbonyl groups. Preferred among such M groups are those containing 1, 2, 3, 4, 5 or 6, especially 1, 2 or 3, hydroxy groups, e.g.

—CONH—CH_2CH_2OH
—CONH—$CH_2CHOHCH_2OH$
—CONH—$CH(CH_2OH)_2$
—$CON(CH_2CH_2OH)_2$

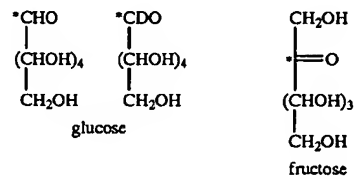
as well as other groups such as

—$CONH_2$
—$CONHCH_3$
—$OCOCH_3$
—$N(COCH_3)H$
—$N(COCH_3)C_{1-3}$-alkyl
—$N(COCH_3)$-mono, bis or tris-hydroxy C_{1-4}-alkyl
—$N(COCH_2OH)$-mono, bis or tris-hydroxy C_{1-4}-alkyl
—$N(COCH_2OH)_2$
—$CON(CH_2CHOHCH_2OH)(CH_2CH_2OH)$
—CONH—$C(CH_2OH)_3$ and
—CONH—$CH(CH_2OH)(CHOHCH_2OH)$.

In general, the M groups will preferably each comprise a polyhydroxy C_{1-4}-alkyl group, such as C_{1-4}-alkyl groups substituted by 1, 2, 3 or 4 hydroxy groups (e.g. hydroxymethyl, 2-hydroxyethyl, 2,3-bishydroxy-propyl, 1,3-bishydroxyprop-2-yl, 2,3,4-trihydroxybutyl, and 1,2,4-trihydroxybut-2-yl) optionally connected to the phenyl ring via a CO, SO or SO_2 group (e.g. $COCH_2OH$ or SO_2CH_2OH).

Preferred compounds are those in which two or three non-adjacent R groups in the or each C_6R_5 moiety are iodine and at least one, and preferably two or three, R groups in the or each C_6R_5 moiety are M or M_1 moieties; each M independently is a non-ionic hydrophilic moiety; and each M_1 independently represents a C_{1-4}-alkyl group substituted by at least one hydroxyl group and optionally linked to the phenyl ring via a carbonyl, sulphone or sulphoxide group, at least one 2 group, preferably at least two R groups and especially preferably at least one R group in the or each C_6R_5 moiety, being an M_1 moiety. Especially preferred are the compounds disclosed in WO-A-96/09282.

(3) sugars:



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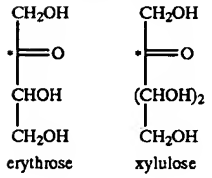
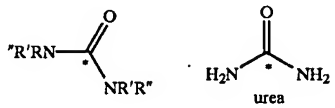


erythrose xylulose

(4) ketones:

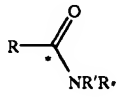
(wherein R and R' are as defined above)

(5) ureas:

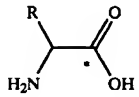


urea

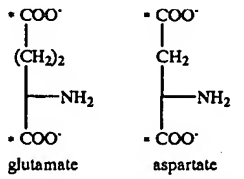
(6) amides:



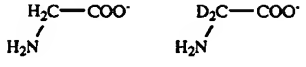
(7) amino acids:



and peptides and proteins labelled in the carbonyl position, particularly those known in the art to be useful for targetting tumour cells. Of the proteins, albumin is especially preferred. Polymers are also useful, particularly those with low toxicity (eg, polylysine) and those with many carboxyl groups (eg polyglutamic acid). The following amino acids are especially preferred:



glutamate aspartate



glycine

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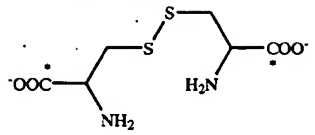
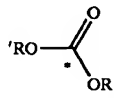
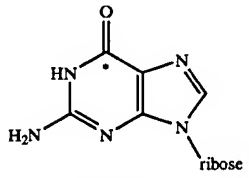


cystine

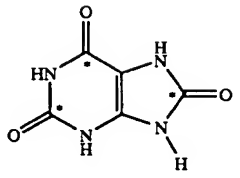
(8) carbonates:



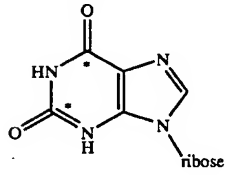
(9) nucleotides:



guanylic acid

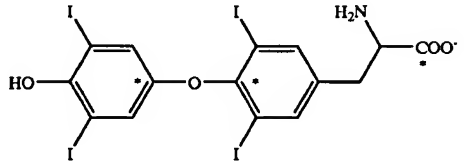


uric acid

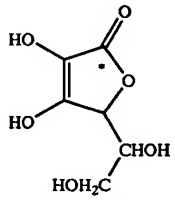


xanthylic acid

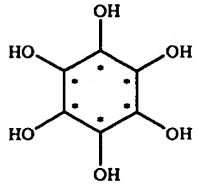
(10) tracers:

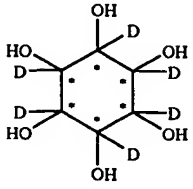


thyroxine



ascorbic acid





inositol

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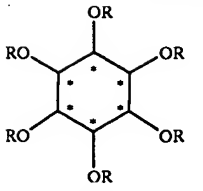
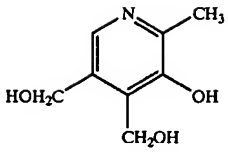
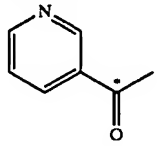


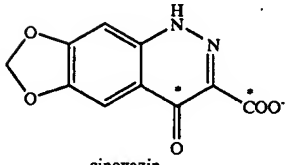
inositolhexanicotinate
(hexanicil, Astra)



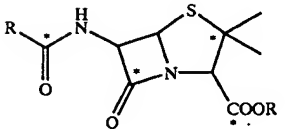
pyridoxine

R =

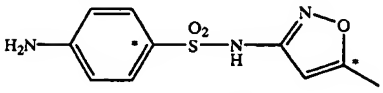




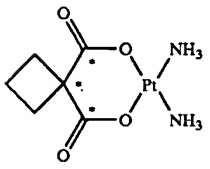
cinoxazin



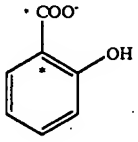
penicillin derivatives



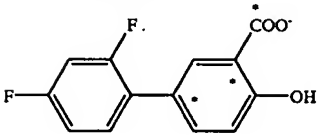
Sulfonamide



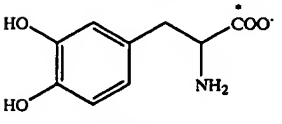
Carboplatinum



sallcylate

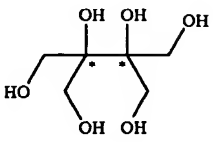


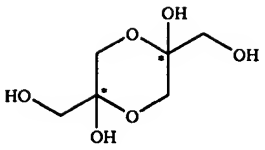
diflunisal

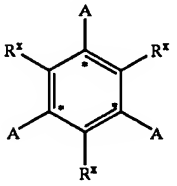


DOPA

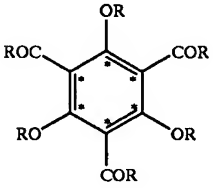
and
(11) compounds such as:

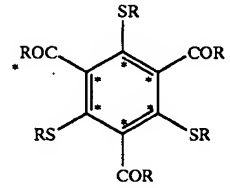


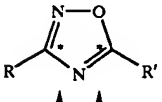


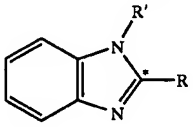


(wherein R denotes any of the conventional side chains suitable for use in X-ray contrast agents and A denotes I, D, OR, RC=O or ^{19}F)

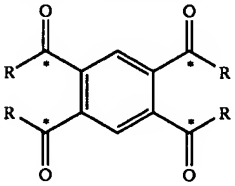


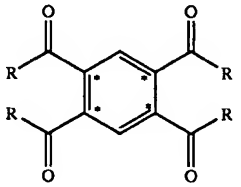


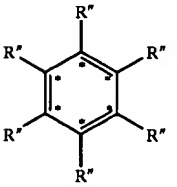


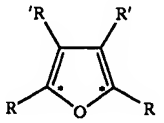


(chose one of the two ^{13}C)



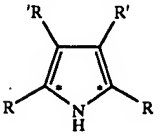


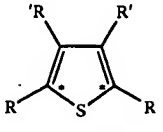


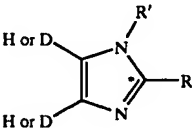


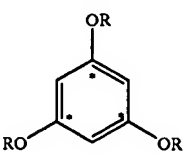
R" not N, H, F, P

R not H, F, P









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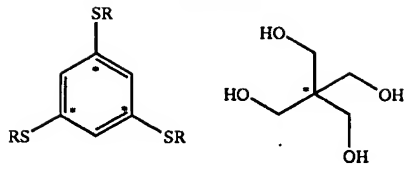


In any of the above definitions, unless otherwise specified R, R', R" and R'" denote any suitable substituent, preferably a substituent bound by a non-magnetic nucleus.

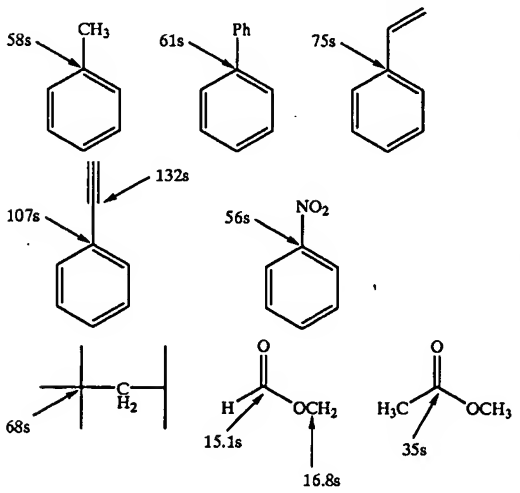
The partly or wholly deuterated or ^{14}F analogues of any of these compounds are particularly preferred.

Certain of the above-mentioned ^{13}C enriched compounds are novel per se and form a further aspect of the invention. Compounds which are water soluble are particularly preferred.

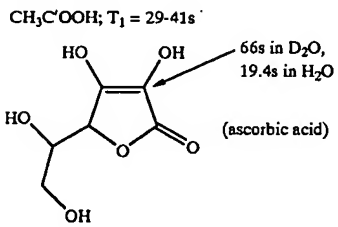
In general, ^{13}C enriched amino acids and any known contrast agents from the fields of X-ray contrast agents and MRI contrast agents (the chelating agent without the metal counterion eg conventional Gd chelating agents without Gd) are preferred as high T_1 agents Intermediates in normal metabolic cycles such as the citric acid cycle eg. fumaric acid and pyruvic acid are preferred for the imaging of metabolic activity.

T_1 values for ^{13}C enriched compounds useful in the invention are reported in the literature or may be routinely determined. Examples include:

(a) non-water soluble (i.e soluble in an organic solvent)



(b) water soluble



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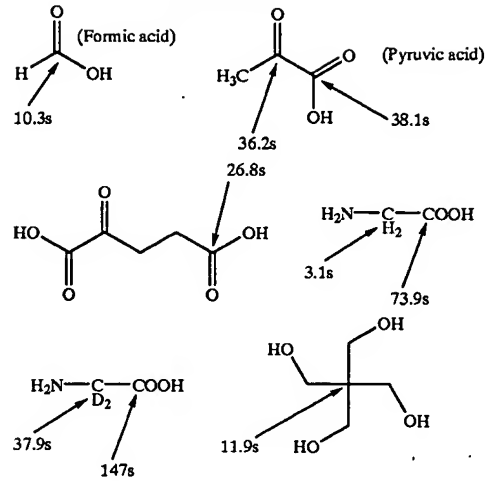

Hyperpolarisation may be carried out by any known method and by way of example three such methods are described hereinbelow. It is envisaged that, in the method according to the invention, the level of polarisation achieved should be sufficient to allow the hyperpolarised solution of the high T_1 agent to achieve a diagnostically effective contrast enhancement in the sample to which it is subsequently administered in whatever form In general, it is desirable to achieve a level of polarisation which is at least a factor of 2 or more above the field in which MRI is performed, preferably a factor of 10 or more, particularly preferably 100 or more and especially preferably 1000 or more, eg. 50000.

In a first embodiment of the method according to the invention, hyperpolarisation of the MR imaging nuclei is effected by an OMRI contrast agent. In this embodiment, step (i) of the method comprises:

(a) bringing an OMRI contrast agent and a high T_1 agent into contact in a uniform magnetic field (the primary magnetic field B_o);

(b) exposing said OMRI contrast agent to a first radiation of a frequency selected to excite electron spin transitions in said OMRI contrast agent; and

(c) dissolving in a physiologically tolerable solvent said high T_1 agent. It is preferred that the OMRI contrast agent and high T_1 agent are present as a composition during polarisation.

Dynamic nuclear polarisation may be attained by three possible mechanisms: (1) the Overhauser effect, (2) the solid effect and (3) thermal mixing effect (see A. Abragam and M. Goldman, Nuclear Magnetism: order and disorder, Oxford University Press, 1982). The Overhauser effect is a relaxation driven process that occurs when the electron-nucleus interaction is time-dependent (due to thermal motion or relaxation effects) on the time scale of the inverse electron Larmor frequency or shorter. Electron-nuclear cross-relaxation results in an exchange of energy with the lattice giving rise to an enhanced nuclear polarisation. The overall enhancement depends on the relative strength of the scalar and dipolar electron-nuclear interaction and the microwave power. For static systems both thermal mixing and the solid effect are operative. In the solid effect, the electron spin system is irradiated at a frequency that corresponds to the sum or difference of the electronic and nuclear Larmor frequencies. The nuclear Zeeman reservoir absorbs or emits the energy difference and its spin temperature is modified,

resulting in an enhanced nuclear polarisation. The efficiency depends on the transition probabilities of otherwise forbidden transitions that are allowed due to the mixing of nuclear states by non-secular terms of the electron-nuclear dipolar interaction. Thermal mixing arises when the electron-electron dipolar reservoir establishes thermal contact with the nuclear Zeeman reservoir. This takes place when the characteristic electronic resonance line width is of the order of the nuclear Larmor frequency. Electron-electron cross relaxation between spins with difference in energy equal to the nuclear Zeeman energy is absorbed or emitted by the electronic dipolar reservoir, changing its spin temperature and the nuclear polarisation is enhanced. For thermal mixing both the forbidden and the allowed transitions can be involved.

In the first embodiment where the polarising agent is an OMRI contrast agent, the method may be conveniently carried out by using a first magnet for providing the polarising magnetic field and a second magnet for providing the primary magnetic field for MR imaging. The same magnet could be used for both purposes. FIG. 1 of the accompanying drawings is a schematic representation of an apparatus suitable for carrying out the first embodiment of the invention. A freestanding polarising magnet (1) optionally together with a filter surrounds an EPR resonator (2) which provides the nuclear polarisation. A container (3) comprising a pump is provided for carrying the contrast composition which is delivered to a subject (4) by a delivery line (5). The subject is situated within a conventional MR scanner (6).

In the above apparatus, a dielectric resonator may be used in the dynamic nuclear polarisation process. Generally speaking, dynamic nuclear polarisation requires a volume with a fairly strong high frequency magnetic field and an accompanying electric field which is made as small as possible. A dielectric resonator may be used to provide a preferred field arrangement in which the magnetic field lines are shaped like a straw in a sheaf of corn with an electric field forming circles like the thread binding the sheaf. A field arrangement of this type may be formed by one of several rings or tubes of a material with a high dielectric constant and low loss. The man skilled in the art will appreciate that such a tube will exhibit different electromagnetic resonant modes. One of the dominant modes has the desired characteristic of electric field circulating around the tube axis within the wall and being zero at the axis and everywhere perpendicular to it. The magnetic field on the other hand is concentrated around the tube axis and mainly directed along it. The composition to be polarised is conveniently placed inside the resonator which is itself placed inside a metal box with a clearance typically of the order of the size of the resonator, and is excited to the desired resonance with a coupling loop or the like. The metal box ensures that the electromagnetic energy does not leak away by radiation. FIG. 2 of the accompanying drawings shows a dielectric resonator (1) (with an axis of rotational symmetry (2)) within a metal box (3).

An alternative to the dielectric resonator is a resonant cavity of which several are known to those skilled in the art. One simple and efficient resonant cavity is a metal box, such as a cylindrical metal box. A suitable mode is the one known as TM1,1,0 which produces a perpendicular magnetic field on the axis of the cavity. It is possible to excite two such modes in the same cavity at the same frequency producing fields which are mutually perpendicular. By arranging them to have a 90° phase difference a rotating field can be produced which is especially efficient for implementing dynamic polarisation with a minimum of dissipation in the sample. Modes with similar field distributions for different shapes of cavities e.g. rectangular cavities are familiar to those skilled in the art.

The composition may also be dispersed into a plurality of compartments during the dynamic nuclear polarisation step. Thus the composition might be typically divided into parallel channels provided, for example, by parallel separating plates, discs or tubes, typically open-ended tubes. The electric losses (eddy currents) in the composition caused by the magnetic field are decreased by dividing the composition into smaller volumes using electrically isolating barriers, preferably situated perpendicular to the field. If the composition is in a cylindrical vessel surrounded by a dielectric resonator as described hereinbefore, the isolating barriers would be planes passing radially from the vessel axis to its wall. A simpler and more practical arrangement is to polarise the composition in a container which contains a plurality of thin-walled tubes of an isolating material such as quartz, glass or plastic. This has the advantage of reducing the electric losses in the composition which allows a larger volume of composition to be polarised for the same applied electromagnetic power. The walls, the inner, outer or both of the tubes may similarly serve as the substrate onto which the OMRI contrast agent is bound so that pressure applied to one end of the container may force the polarized, substantially OMRI contrast agent free, fluid high T_1 agent from the container, for example with a delivery line leading to the subject (patient) undergoing MR examination.

It is envisaged that in the first embodiment of the method according to the invention, use may be made of any known OMRI contrast agent capable of polarising a high T_1 agent to an extent such that a diagnostically effective contrast enhancement, in the sample to which the high T_1 agent is administered, is achieved. Where the OMRI contrast agent is a paramagnetic free radical, the radical may be conveniently prepared in situ from a stable radical precursor by a conventional physical or chemical radical generation step shortly before polarisation, or alternatively by the use of ionising radiation. This is particularly important where the radical has a short half-life. In these cases, the radical will normally be non-reusable and may conveniently be discarded once the separation step of the method according to the invention has been completed.

In solids, it is preferred to effect dynamic nuclear polarisation by irradiating an electron spin at low temperature and high field. Specific examples of dynamic nuclear polarisation of solid high T_1 agents are:

(1) 15N-Ala labelled T4-lysosome and 13C-Glycine in frozen aqueous solutions of 60:40 glycerol/water with the free radical 4-amino TEMPO as the source of electron polarisation (D. A. Hall, D. Maus, G. Gerfen and R. G. Griffin, Science, 1997), Enhancements of ca. 50 and 100 were obtained, respectively, at 5 T and 40 K;

(2) Carboxy-13C labelled glycine in frozen aqueous solution of 60:40 glycerol/water with TEMPO as the free radical. An enhancement of 185 at 5 T and 14 K was obtained (G. J. Gerfen, L. R. Becerra, D. A. Hall, R. G. Griffin, R. J. Temkin, D. J. Singel, J. Chem. Phys. 102(24), 9494–9497 (1995);

(3) Dynamic polarisation of protons and deuterons in 1,2-ethanediol doped with complexes of Cr at 2.5 T. The obtained degree of polarisation is 80% (W. De Boer and T. O Niinikoski, Nucl. Instrum. Meth. 114, 495 (1974).

Preferably of course a chosen OMRI contrast agent will exhibit a long half-life (preferably at least one hour), long

relaxation times (T_{1e} and T_{2e}), high relaxivity and a small number of ESR transition lines. Thus the paramagnetic oxygen-based, sulphur-based or carbon-based organic free radicals or magnetic particles referred to in WO-A-88/10419, WO-A-90/00904, WO-A-91/12024, WO-A-93/02711 or WO-A-96/39367 would be suitable OMRI contrast agents.

However, OMRI contrast agents useful in the first embodiment of the present method are not limited to paramagnetic organic free radicals. Particles exhibiting the magnetic properties of paramagnetism, superparamagnetism, ferromagnetism or ferrimagnetism may also be useful OMRI contrast agents, as may be other particles having associated free electrons. Superparamagnetic nanoparticles (eg. iron or iron oxide nanoparticles) may be particularly useful. Magnetic particles have the advantages over organic free radicals of high stability and a strong electronic/nuclear spin coupling (i.e. high relaxivity) leading to greater Overhauser enhancement factors.

For the purposes of administration, the high T_1 agent should be preferably administered in the absence of the whole of, or substantially the whole of, the OMRI contrast agent. Preferably at least 80% of the OMRI contrast agent is removed, particularly preferably 90% or more, especially preferably 95% or more, most especially 99% or more. In general, it is desirable to remove as much OMRI contrast agent as possible prior to administration to improve physiological tolerability and to increase T_1. Thus preferred OMRI contrast agents for use in the first embodiment of the method according to the invention are those which can be conveniently and rapidly separated from the polarised high T_1 MR imaging agent using known techniques as discussed below. However where the OMRI contrast agent is non-toxic, the separation step may be omitted. A solid (eg. frozen) composition comprising an OMRI contrast agent and a high T_1 agent which has been subjected to polarisation may be rapidly dissolved in saline (eg. warm saline) and the mixture injected shortly thereafter.

In the separation step of the first embodiment of the method of the invention, it is desirable to remove substantially the whole of the OMRI contrast agent from the composition (or at least to reduce it to physiologically tolerable levels) as rapidly as possible. Many physical and chemical separation or extraction techniques are known in the art and may be employed to effect rapid and efficient separation of the OMRI contrast agent and high T_1 agent. Clearly the more preferred separation techniques are those which can be effected rapidly and particularly those which allow separation in less than one second. In this respect, magnetic particles (eg. superparamagnetic particles) may be advantageously used as the OMRI contrast agent as it will be possible to make use of the inherent magnetic properties of the particles to achieve rapid separation by known techniques. Similarly, where the OMRI contrast agent or the particle is bound to a solid bead, it may be conveniently separated from the liquid (i.e. if the solid bead is magnetic by an appropriately applied magnetic field).

For ease of separation of the OMRI contrast agent and the high T_1 agent, it is particularly preferred that the combination of the two be a heterogeneous system, eg. a two phase liquid, a solid in liquid suspension or a relatively high surface area solid substrate within a liquid, eg. a solid in the form of beads fibres or sheets disposed within a liquid phase high T_1 agent. In all cases, the diffusion distance between the high T_1 agent and OMRI contrast agent must be small enough to achieve an effective Overhauser enhancement. Certain OMRI contrast agents are inherently particulate in nature, eg. the paramagnetic particles and superparamagnetic agents referred to above. Others may be immobilized on, absorbed in or coupled to a solid substrate or support (eg. an organic polymer or inorganic matrix such as a zeolite or a silicon material) by conventional means. Strong covalent binding between OMRI contrast agent and solid substrate or support will, in general, limit the effectiveness of the agent in achieving the desired Overhauser effect and so it is preferred that the binding, if any, between the OMRI contrast agent and the solid support or substrate is weak so that the OMRI contrast agent is still capable of free rotation. The OMRI contrast agent may be bound to a water insoluble substrate/support prior to the polarisation or the OMRI contrast agent may be attached/bound to the substrate/support after polarisation. The OMRI contrast agent may then be separated from the high T_1 agent e.g. by filtration before administration. The OMRI contrast agent may also be bound to a water soluble macromolecule and the OMRI contrast agent-macromolecule may be separated from the high T_1 agent before administration.

Where the combination of an OMRI contrast agent and high T_1 agent is a heterogeneous system, it will be possible to use the different physical properties of the phases to carry out separation by conventional techniques. For example, where one phase is aqueous and the other non-aqueous (solid or liquid) it may be possible to simply decant one phase from the other. Alternatively, where the OMRI contrast agent is a solid or solid substrate (eg. a bead) suspended in a liquid high T_1 agent the solid may be separated from the liquid by conventional means eg. filtration, gravimetric, chromatographic or centrifugal means. It is also envisaged that the OMRI contrast agents may comprise lipophilic moieties and so be separated from the high T_1 agent by passage over or through a fixed lipophilic medium or the OMRI contrast agent may be chemically bound to a lipophilic solid bead. The high T_1 agent may also be in a solid (eg. frozen) state during polarisation and in close contact with a solid OMRI contrast agent. After polarisation it may be dissolved in heated water or saline or melted and removed or separated from the OMRI contrast agent where the latter may be toxic and cannot be administered.

One separation technique makes use of a cation exchange polymer and a cationic OMRI contrast agent, eg. a triarylmethyl radical carrying pendant carboxylate groups. Alternatively acidifying the solution to around pH 4 may cause the OMRI contrast agent to precipitate out. Separation may then be carried out for example by filtration followed by neutralisation. An alternative technique involves adding ions which causes precipitation of ionic OMRI agents which may then be filtered off.

Certain OMRI contrast agents, such as the triarylmethyl radical, may have an affinity for proteins. Thus, after polarisation, a composition containing an OMRI contrast agent with a protein affinity may be passed through or over a protein in a form which exposes a large surface area to the agent eg. in particulate or surface bound form. In this way, binding of the OMRI contrast agent to the protein enables it to be removed from the composition.

Alternatively when a hydrophilic high T_1 agent is in a solid (eg. frozen) form it may be brought into contact with a hydrophobic OMRI contrast agent which is dissolved in an organic fluid with a melting temperature higher than the high T_1 agent. The mixture is frozen and polarisation performed. After polarisation, the mixture is heated and the solid OMRI contrast agent and its solvent are removed. The high T_1 agent will remain hyperpolarised for a significant time in the frozen state and may be transported long distances before being dissolved in water or saline for injection.

In a second embodiment of the method according to the invention, hyperpolarisation of the nuclei is effected by a hyperpolarisable gas. In this second embodiment, step (i) of the method according to the invention comprises:

(a) hyperpolarising a hyperpolarisable gas before, during or after introducing a high T_1 agent thereto whereby to cause nuclear polarization of said high T_1 agent;

(b) dissolving in a physiologically tolerable solvent said high T_1 agent,

and wherein said high T_1 agent is not limited to ^{13}C enriched agents at one or more carbonyl or quaternary carbon positions

By hyperpolarisable gas is meant a gas with a non-zero spin angular momentum capable of undergoing an electron transition to an excited electron state and thereafter of decaying back to the ground state.

Depending on the transition that is optically pumped and the helicity of the light a positive or negative spin hyperpolarisation may be achieved (up to 100%). Examples of gases suitable for use in the second embodiment of the method of the invention include the noble gases He (eg. ^3He or ^4He) and Xe (eg. ^{129}Xe) preferably He, particularly preferably ^3He. Alkali metal vapours may also be used eg. Na, K, Rb, Cs vapours. Mixtures of the gases may also be used or the hyperolarisable gas may be used in liquid or solid form. The term hyperpolarisable gas also covers any gas with non-zero nuclear spin which may be polarised by optical pumping and is preferably ^{129}Xe or ^3He.

It will be appreciated that in the second embodiment of the invention, the hyperpolarised gas may transfer polarisation to the nuclear spin system of a high T_1 agent directly or indirectly. Where the high T_1 agent is to be polarised indirectly by water vapour, it may be advantageously water soluble.

For the purposes of polarisation according to the second embodiment of the invention, the high T_1 agent may be generally in gaseous, liquid or solid form.

Where the high T_1 agent is polarised whilst in a gaseous state, it is convenient (for the purposes of separation from the hyperpolarised gas and of administration) to be able to rapidly convert it into a liquid or solid. This has the added benefit of significantly increasing T_1. Thus removing the elevated pressure and temperature imposed on the gas mixture will lead to rapid cooling and condensation. Yet further cooling is possible by, for example, contacting the polarised high T_1 agent with a cold surface.

In a preferred embodiment, a hyperpolarised fluid eg. ^{129}Xe at elevated pressure and/or low temperature is passed through a column of solid ^{13}C enriched and/or ^{19}F enriched high T_1 agent until steady state polarisation of the solid is almost achieved. In general any of the above-mentioned ^{19}C enriched agents may be used.

In another preferred embodiment, a hyperpolarised gas is frozen/crystallised on the solid/frozen surface of a solid high T_1 agent which has been prepared with as large a surface area as possible. The mixture may be transported before warm administrable media (eg. saline) is added and physiological temperature reached before injection.

^{129}Xe gas can be produced in a highly spin polarised state in macroscopic quantities. Due to the limited solubility and inert nature of xenon there is interest in transferring the polarisation to other nuclei.

It can also be produced by irradiating a polarising agent, e.g. with an electron spin resonance transition stimulating radiation (e.g. microwave radiation). This forms a further aspect of the invention. Viewed from this aspect the invention provides a method of magnetic resonance investigation of a sample, preferably of a human or non-human animal body, said method comprising:

i) producing solid hyperpolarised ^{129}Xe by irradiating a polarising agent whereby to cause dynamic nuclear polarisation.

In the above method said polarising agent is preferably a substance containing an unpaired electron, for example nitroxide, trityl, Cr(V), or the OMRI agents mentioned above.

Considerable interest has been generated in the novel technique of MR lung imaging using hyperpolarised gases such as ^3He and ^{129}Xe as inhaled contrast media. However, the production of these gases in their hyperpolarised form is labourious and time consuming. At the present time, ^3He, where most of the interest is today, can be generated at a rate of a few liters an hour. However, if the hyperpolarisation could be done in the liquid or solid phase, much higher production rates would be possible. Using only "brute force", i.e. milliKelvin temperatures and >10 T, fields would be an extremely costly method, however, "double brute force", i.e. irradiation of frozen Xe in the presence of a free radical (metal ion, trityl radical, nitroxide, etc.) at a comparatively moderate temperature (a few K) would be a more practical method. The radical would be added either in pure form or bound to a matrix. After the irradiation had been carried out, heating of the sample would release the hyperpolarised gas and a new batch of Xe could be condensed and irradiated. Since the hyperpolarisation in this case is carried out on solid Xe, the possibilities of producing large amounts of gas would be considerable.

The main relaxation mechanism for solid ^{129}Xe is spin exchange with the rapidly relaxing ^{131}Xe, the major component in natural xenon. The magnetogyric ratio of ^{129}Xe and ^{131}Xe differs by a factor of four. Normally the line widths of the resonances of solids are on the order of a few kHz. When the difference in Larmor frequency is on the same order as the line width, the polarisation of the nuclei will rapidly equilibrate. Assuming that we have a cold (colder than the freezing point of xenon, around 150 K, depending on the pressure), finely divided (some micrometers grain size), sample of a ^{13}C labelled substance with a long T_1 in the solid and allow hyperpolarised xenon to form frost on the powder. If this operation is performed in a magnetic field of suitable strength the ^{129}Xe and the 13C will overlap and Xe-C spin flip-flops will be efficient, equilibrating the polarisation between xenon and carbon. The xenon can then be pumped off and the process repeated until a suitable level of polarisation is achieved. What the suitable field strength is depends on the exact lineshapes but assuming line widths on the order of 5–10 kHz, which is quite normal for solids, the optimum field is around 10 mT, typically the field on the outside of an NMR-magnet or a small toy magnet. The basis for this is that the centre frequency of the line is field dependent whereas the linewidth is essentially independent of the field.

FIG. 3 shows the behaviour of such a system at various field strengths. One important factor to take into account is that all the nuclei in the sample must be taken into consideration. This method will work for transfer from ^{129}Xe to ^{13}C and possibly to ^{29}Si but it is not expected to work with ^{19}N which has a resonance frequency that is closer to ^{131}Xe than to ^{129}Xe. There will be interference from quadrupolar nuclei like ^{23}Na, ^{79}Br, ^{81}Br, ^{127}I and a number of transition metals, all having resonance frequencies similar to carbon.

In order to generate a hyperpolarised gas, the gas is first subjected to a discharge or other means of excitation (eg. an appropriate radiofrequency) which creates a metastable

unpaired electron spin state and is then optically (eg. laser) pumped at an appropriate frequency to create electron hyperpolarisation. The various methods for achieving this are well known to those skilled in the art or are described in inter alia U.S. Pat. No. 5545396.

Preferred hyperpolarisable gases for use in the second embodiment of the method according to the invention are those which can be conveniently and rapidly separated from the polarised high T_1 agent. Noble gases are particularly useful given their very low boiling points and inertness. Preferably the chosen gas will exhibit a long hyperpolarisability half-life (preferably at least 1000 s, particularly preferably at least 4000 s and especially preferably 8000 s or more).

A hyperpolarised gas may, if desired, be stored for extended periods of time in a hyperpolarised state. This is achieved by maintaining the gas at very low temperatures, preferably in a frozen state.

For ease of separation of the hyperpolarisable gas and the high T_1 agent, the combination of the two may be advantageously a heterogeneous system, eg. the high T_1 agent is a solid at ambient temperatures. In all cases, the diffusion distance between the high T_1 agent and gas, fluid or solid must be small enough to achieve an effective polarisation.

In the separation step of the second embodiment of the method of the invention, it is desirable to remove substantially the whole of the hyperpolarisable gas from the composition (or at least to reduce it to physiologically tolerable levels) as rapidly as possible. If desired, the gas may be reused which may be an important consideration given the expense of noble gases. Many physical and chemical separation or extraction techniques known in the art may be employed to effect rapid and efficient separation of the hyperpolarisable gas and high T_1 agent. Clearly the more preferred separation techniques are those which can be effected rapidly and particularly those which allow separation in a fraction of the relaxation time T_1 of the high T_1 agent.

In a third embodiment of the method of the invention, hyperpolarisation of the MR imaging nuclei is effected by the use of a high field as described in U.S. Pat. No. 5,479,925 (GEC) and U.S. Pat. No. 5,617,859 (GEC). U.S. Pat. No. 5,479,925 discloses a method for generating MR angiograms in which a contrast agent is passed through a small, high field polarising magnet ex vivo, in order to generate a high longitudinal magnetisation in the agent prior to its administration to the subject. There is however no mention or suggestion of the use of high T_1 agents to achieve an improved effect.

Generally speaking, polarisation of an MR imaging nuclei may be achieved by thermodynamic equilibration at low temperature and high magnetic field. Where the contrast medium to be administered is a solid material (e.g. crystalline), it may be introduced into a magnetic field at very low temperature. Under these conditions, T_1 is very long (typically many hours or months) and consequently it takes an unacceptably long time for the medium to reach thermodynamic equilibrium. Thus if the contrast medium undergoes small movements in the gradient field for example by exposure to a magnetic field gradient and ultrasound or by relative movement within the gradient field, T_1 will drop. When thermodynamic equilibrium is attained, all nuclei in the contrast medium will be highly polarised relative to room temperature and to normal magnetic fields used in MRI. This procedure has the advantage of allowing the contrast medium to be removed from the magnet and transported in a "ready-to-use" form to the place where it is to be used. Preferably but not essentially transport may take place at a relatively low temperature (e.g. at liquid nitrogen temperature). The T_1 of the high T_1 solid contrast medium will be long enough to allow transport at ambient temperature before use.

One of the main obstacles in using so-called 'brute force' polarisation as a method for hyperpolarising samples are the long T_1 values at low temperatures and high fields, typically several weeks at temperatures below 1 K. However it has been found that it is possible to utilise the non-linear field dependence of T_1 to shorten the time necessary for relaxation by a gradual increase of the external magnetic field.

As stated above, it is of great interest to obtain hyperpolarised injectable contrast agents. Theoretically, the simplest way of obtaining a highly spin-polarised material is to cool it to a very low temperature in a strong magnetic field and let the sample reach thermal equilibrium. The major practical problem in using this technique is the time required for the thermal equilibration to occur. At temperatures below 1 K the time constant for that process, T_1, might beon the order of weeks.

The time constant of nuclear longitudinal relaxation, T_1, shows a quadratic dependence on the field strength in solid materials:

$$T_1=T_{1,0}+cB$$

Where $T_{1,0}$ is the time constant for relaxation at no external magnetic field, c is a constant, and B is the external magnetic field.

The rate of magnetisation of the sample, dM/dt, at a given field strength will then be given by:

$$dM/dt=(M_{max}-M)/T_1$$

Where M_{max} is the magnetisation of the sample after complete relaxation at the final field. Since the field-dependence of the time constant is non-linear, it will be possible to obtain a larger magnetisation at a given time by constantly tuning the external magnetic field so that the rate of magnetisation all the time is as big as possible. The example shown in attached FIGS. **4** and **5** was chosen to simulate the behaviour of the carbonyl carbon in solid sodium acetate. The T_1 at 7 T is 1700 seconds and the $T_{1,0}$ is about 5 seconds. The time to reach the same degree of magnetisation as after 1700 seconds at a constant field of 7 T, is reduced to 1390 seconds, a reduction of almost 20%, which could easily reduce the equilibration time by one week at milliKelvin temperatures. The optimised field-ramp is shown in FIG. **4**, whilst FIG. **5** shows the expected values from a numerical integration of the equation for dM/dt given above. This process will be applicable for all nuclei with spin but will be most interesting with compounds with long T_1 values

As stated above, one of the main obstacles in using so-called 'brute force' polarisation as a method for hyperpolarising samples is the long T_1 values found at low temperatures and high fields, typically several weeks at temperatures below 1 K. It is possible to use the technique of low-field matching to increase the relaxation rate and the degree of polarisation of the nuclear spins in solids at low temperature. This has the additional advantage that a brute force polariser does not need to possess any radio frequency electronics.

It is well known that different nuclei in the same molecule will relax with different time constants. A way of speeding up the polarisation of the interesting ^{13}C nucleus and at the same time obtaining a better polarisation is to use cross-

polarisation from the quickly relaxing proton to the slowly relaxing carbon, a method routinely used in solid-state NMR spectroscopy. Due to the big difference in magnetogyric ratio between the proton and ^{13}C, the energy difference is large and hence the polarisation transfer slow. The magnetogyric ratio of the proton is roughly a factor of four larger than that of carbon. The situation can be improved by utilising the procedure of spin locking under Hartman-Hahn conditions. Spin-lock (90_x-long pulse,) at both nuclei with the amplitude (B_1) of the long pulse satisfying the Hartman-Hahn condition:

$$\gamma H B_{1H} = \gamma C B_{1C}$$

where γH is the magnetogyric ratio of hydrogen, γC is the magnetogyric ratio of carbon, B_{1H} is the proton excitation field and B_{1C} is the carbon excitation field.

This allows for mutually matched flip-flops of the spins. Since this is a spin-spin process, it usually occurs on time scales from about 100 μs to a few ms.

One problem with this is that radiofrequency electronics are required and furthermore the homogeneity of the magnetic field must be high enough to allow precise pulse angles. A way to circumvent this problem is the following.

A crude way of stating the Hartman-Hahn condition is to say that spin diffusion is efficient when the resonance lines of the two nuclei overlap. Assume the substrate to be a solid material with a half-height line width of 5 kHz. This linewidth is caused by dipolar coupling and is independent of the external field. The Hartman-Hahn condition is now restated as follows. Efficient spin-diffusion takes place when the maxima of the two resonances are separated by less than the sum of their half-height line-widths. The field where this condition is fulfilled is derived as follows.

The resonance frequency, ν is given by:

$$\nu = \gamma B_0 / 2\pi \quad (1)$$

where γ is the magnetogyric ratio, and B_0 is the external magnetic field. The required separation, ν, was 5 kHz:

$$\nu = \nu_H - \nu_C = 5000\ s^{-1} \quad (2)$$

Combination of equation (1) and (2) gives:

$$\nu = B_0(\gamma_H - \gamma_C)/2\pi$$

which can be rewritten as:

$$B_0 = \nu 2\pi/(\gamma_H - \gamma_C) = 156\ \mu T$$

This field is within a factor 3 of the earth's magnetic field which means that if the sample is removed from the polarising magnet for a few seconds the polarisation will equilibrate between carbon and hydrogen on a time scale similar to T_2 so there will be plenty of time to put the sample back into the magnet again before the T_1 relaxation becomes significant, even if it has to be kept in mind that the T_1 shortens dramatically for solids in low fields. It will, however, never be as short as the T_2.

This procedure can be repeated after the protons have repolarised again, successively building up the carbon polarisation until the spin-temperature of the two nuclei become the same. The protons in solid sodium acetate has, at room temperature, a T_1 of 31 s whilst the T_1 of the carbonyl carbon in the same sample is 1700 s. If this difference could be used completely there would be a shortening of the polarisation time by a factor of 55. Generally fluorine relaxes even faster than protons and it would be possible to include a fluorine atom in the contrast agent molecule as an internal relaxation agent.

It is also possible to use quadrupolar nuclei for this process. The sodium ions in solid sodium acetate have a T_1 of 1.7 s at room temperature. Sodium has a magnetogyric ratio only slightly higher than carbon which means that equilibration occurs at a much higher field, in this case at 8.9 mT, a field found about 15 cm above the Dewar of a 7 T NMR magnet. This is of great help for rapid polarisation of samples at low temperature, but is also a problem in the storage of polarised samples. The magnetic storage field must be large enough to avoid overlap of the resonances of the wanted nucleus and any rapidly relaxing quadrupolar nuclei. It is known that this phenomenon causes the rapid relaxation of frozen ^{129}Xe in a low field, when spin diffusion to the quadrupolar ^{131}Xe becomes efficient.

There is also the possibility of transferring polarisation from unpaired electrons to carbon. Due to the great difference in magnetogyric ratios this requires a field much lower than the earth magnetic field to become efficient. Such a low field requires that the sample be moved to a magnetically shielded area. One way of achieving this is to have a small magnet with opposite polarity some distance along the polarity axis of the main coil. With careful design the fields can be made to cancel in the centre of the small magnet.

The magnetic field strength used in this third embodiment of the invention should be as high as possible, preferably >1 T, more preferably 5 T or more, especially preferably 15 T or more. The temperature should be very low e.g. 100 K or less, preferably 4.2 K or less, more preferably 1 K or less, even more preferably 0.1 K or less, especially preferably 1 mK or less.

Thus viewed from a further aspect the present invention provides a method for preparing polarised high T_1 agents, said method comprising the polarisation stages of:

(a) subjecting a high T_1 agent to a high magnetic field (e.g. 1 T or more) at low temperature (e.g. 100 K or less);

(b) exposing the agent to a T_1 shortening effect in order to attain thermodynamic equilibrium at said low temperature.

The T_1 shortening effect may be provided by exposure to a variable magnetic field gradient but it may also be achieved by adding magnetic material (e.g. paramagnetic, superparamagnetic or ferromagnetic materials) to the agent during the period when the agent is exposed to low temperature, field cycling to a field allowing cross polarisation, gradually increasing the magnetic field at such a rate that the increase in polarisation of the high T_1 agent is maximised, gradually decreasing the temperature at such a rate that the increase in polarisation of the high T_1 agent is maximised, or adding a material with unpaired electrons during the period when the high T_1 agent is exposed to said low temperature. Possible T1 shortening agents include Gd and NO but preferred T_1 shortening agents are O_2 and NO which may be conveniently separated from the high T_1 agent before transportation and subsequent use.

In the third embodiment of the invention, both the high T_1 agent and the aqueous solvent (eg. water) in which it is dissolved may be polarised. This may be carried out at low temperature conveniently in the same magnetic field and after mixing the adminstrable composition should be warmed very rapidly prior to administration.

Thus viewed from a further aspect, the present invention provides an administrable composition comprising a polarised high T_1 agent and polarised water.

The high T_1 agents used in the method according to the invention may be conveniently formulated with conven-

tional pharmaceutical or veterinary carriers or excipients. Formulations manufactured or used according to this invention may contain, besides the high T_1 agent, formulation aids such as are conventional for therapeutic and diagnostic compositions in human or veterinary medicine. Thus the formulation may for example include stabilizers, antioxidants, osmolality adjusting agents, solubilizing agents, emulsifiers, viscosity enhancers, buffers, etc. The formulation may be in forms suitable for parenteral (eg. intravenous or intraarterial) or enteral (eg. oral or rectal) application, for example for application directly into body cavities having external voidance ducts (such as the lungs, the gastrointestinal tract, the bladder and the uterus), or for injection or infusion into the cardiovascular system. However solutions, suspensions and dispersions in physiological tolerable carriers will generally be preferred.

For use in in vivo imaging, the formulation, which preferably will be substantially isotonic, may conveniently be administered at a concentration sufficient to yield a 1 micromolar to 10M concentration of the high T_1 agent in the imaging zone; however the precise concentration and dosage will of course depend upon a range of factors such as toxicity, the organ targeting ability of the high T_1 agent and the administration route. The optimum concentration for the MR imaging agent represents a balance between various factors. In general, optimum concentrations would in most cases lie in the range 0.1 mM to 10M, preferably more than 10 mM, especially more than 100 mM. Isotonic solution may be especially preferred. In certain circumstances concentrations above 1M are preferred. Formulations for intravenous or intraarterial administration would preferably contain the high T_1 agent in concentrations of 10 mM to 10M, especially more than 50 mM. For bolus injection the concentration may conveniently be 0.1 mM to 56M, preferably more than 200 mM, more preferably more than 500 mM. In certain circumstances, the preferred concentration is above 1M, even more preferably above 5M.

Parenterally administrable forms should of course be sterile and free from physiologically unacceptable agents, and should have low osmolality to minimize irritation or other adverse effects upon administration and thus the formulation should preferably be isotonic or slightly hypertonic. Suitable vehicles include aqueous vehicles customarily used for administering parenteral solutions such as Sodium Chloride solution, Ringer's solution, Dextrose solution, Dextrose and Sodium Chloride solution, Lactated Ringer's solution and other solutions such as are described in Remington's Pharmaceutical Sciences, 15th ed., Easton: Mack Publishing Co., pp. 1405–1412 and 1461–1487 (1975) and The National Formulary XIV, 14th ed. Washington: American Pharmaceutical Association (1975). The compositions can contain preservatives, antimicrobial agents, buffers and antioxidants conventionally used for parenteral solutions, excipients and other additives which are compatible with the high T_1 agents and which will not interfere with the manufacture, storage or use of the products.

Where the high T_1 agent is to be injected, it may be convenient to inject simultaneously at a series of administration sites such that a greater proportion of the vascular tree may be visualized before the polarization is lost through relaxation.

The dosages of the high T_1 agent used according to the method of the present invention will vary according to the precise nature of the high T_1 agents used, of the tissue or organ of interest and of the measuring apparatus. Preferably the dosage should be kept as low as possible while still achieving a detectable contrast effect. In general, the maximum dosage will depend on toxicity constraints.

The invention is illustrated with reference to the following non-limiting Examples and the accompanying drawings in which:

FIG. 1 is a schematic representation of an apparatus suitable for carrying out the first embodiment of the invention;

FIG. 2 shows a dielectric resonator (1) (with an axis of rotational symmetry (2)) within a metal box (3);

FIG. 3 shows the behaviour of a system at various field strengths;

FIG. 4 shows a build-up of magnetisation versus time; and

FIG. 5 shows magnetic field versus time.

EXAMPLE 1

A high T_1 agent is placed in a chamber at very low temperature (about 4 K). Fluent O_2 is added and crystallised on the surface of the high T_1 agent. In a separate chamber, frozen H_2O is subjected to the same treatment as the high T_1 agent. Both chambers are placed in a strong magnetic field (about 15 T) and the temperature kept low.

When thermodynamic equilibrium is reached, the temperature is increased to about 200 K. The oxygen disappears as a gas. The high T_1 agent and the frozen H_2O are mixed and stored until needed. The temperature is increased and the solution comprising polarised high T_1 agent and hyperpolarised water is injected.

EXAMPLE 2

300 mg of sterile $Na_2{}^{13}CO_3$ or $NaH^{13}CO_3$ is placed inside a 10 ml plastic injection syringe. The gas inside the syringe is enriched with >20% oxygen. The syringe is placed inside a magnet (1–20 T) at a temperature of about 4 K (0.001–5 K) until thermodynamic equilibrium is reached.

The syringe is removed and transported to the subject located in the MRI magnet. 10 ml of sterile Ringers Solution (at 37° C., pH 7.4) is aspirated and injected at a rate of 10 ml/sec immediately after the high T_1 agent has dissolved. 13C MRI is performed using a fast pulse sequence. T_1 in the blood is about 20 s and the distribution of the agent is followed on the MR imager.

EXAMPLE 3

To a sample of sodium acetate (1-^{13}C) is added α, γ-bisphenyl-β-phenylallyl benzene complex (5% w/w). The compounds are milled together to give an intimate mixture, which is transferred to a borosilicate glass ampule. This is then repeatedly evacuated and filled with helium. The last time a pressure of a 200 mbar of helium is left in the ampule, which is then flame sealed.

The sample is polarized by microwaves (70 GHz) for at least one hour at a field of 2.5 T at a temperature of 4.2 K. The progress of the polarization process is followed by in situ NMR (fast adiabatic passage). When a suitable level of polarization has been reached, the ampule is rapidly removed from the polarizer and, while handled in a magnetic Field of no less than 50 mT, cracked open and the contents are quickly discharged and dissolved in warm (40° C.) water.

Experiment 1: This solution is quickly transferred to a spectrometer and ^{13}C spectrum with enhanced intensity is recorded.

Experiment 2: The sample solution is inserted into an MRI machine with ^{13}C capability and a picture with enhanced intensity and contrast is obtained by a single shot technique.

Experiment 3: The solution is quickly injected into a rat and a ^{13}C MRI picture with enhanced intensity and

contrast is obtained, also in this case, by utilization of a single shot technique.

EXAMPLE 4

To a sample of sodium bicarbonate—^{13}C is added $MnCl_2$ (5% w/w). The compounds are milled together to give an intimate mixture, which is transferred to a borosilicate glass ampule. This is then repeatedly evacuated and filled with helium. The last time a pressure of a 200 mbar of helium is left in the ampule, which is then flame sealed.

The sample is polarized by microwaves (70 GHz) for at least 1 hour at a field of 2.5 T at a temperature of 4.2 K. The progress of the polarization process is followed by in situ NMR (fast adiabatic passage). When a suitable level of polarization has been reached, the ampule is rapidly removed from the polarizer and, while handled in a magnetic field of no less than 50 mT, cracked open and the contents are quickly discharged and dissolved in warm (40° C.) water.

Experiment 1: This solution is quickly transferred to a spectrometer and ^{13}C spectrum with enhanced intensity is recorded.

Experiment 2: The sample solution is inserted into an MRI machine with ^{13}C capability and a picture with enhanced intensity and contrast is obtained by a single shot technique.

Experiment 3: The solution is quickly injected into a rat and a ^{13}C MRI picture with enhanced intensity and contrast is obtained, also in this case, by utilization of a single shot technique.

EXAMPLES 5–7

Low-field Pumping of ^{13}C

EXAMPLE 5

A sample of solid 1-^{13}C-2,2,2',2',2",2"-hexadeuterotris (hydroxymethyl)nitromethane was subjected to a magnetic field of 6.56 T at a temperature of 2.5 K for 10 minutes. The sample was then removed from the centre of the magnet to the stray field (7 mT) for a duration 1 s and then returned to the magnet. After another 10 minutes the process was repeated one more. An ^{13}C-NMR spectrum of the solid sample was recorded and the signal was found to be in accordance with thermal equilibrium at 6.56 T and 2.5 K. T_1-values for the ^{13}C atom in 1-^{13}C-2,2,2',2',2",2"-hexadeuterotris(hydroxymethyl)nitromethane

H_2O, air saturated, 37° C., 7 T	95 s
H_2O, degassed, 37° C., 7 T	102 s
Human bloodplasma, 37° C., 7 T	60 s
Solid, 20° C., 7 T	22 min
Solid, -30° C., 7 T	47 min
Solid, 2.5 K, 7 T	55 hours

EXAMPLE 6

A sample of solid 1-^{13}C-1,1-bis (hydroxydideuteromethyl)-2,2,3,3-tetradeuterocyclopropane was subjected to a magnetic field of 6.56 T at a temperature of 2.5 K for 10 minutes. The sample was then removed from the centre of the magnet to the stray field (7 mT) for a duration of 1 s and then returned to the magnet. After another 10 minutes the process was repeated one more. A ^{13}C-NMR spectrum of the solid sample was recorded and the signal was found to be in accordance with thermal equilibrium at 6.56 T and 2.5 K.

EXAMPLE 7

A sample of solid 2-^{13}C-2,2-bis(trideuteromethyl)-1,1,3,3-tetradeuteropropane-1,3-diol was subjected to a magnetic field of 6.56 T at a temperature of 2.5 K for 10 minutes. The sample was then removed from the centre of the magnet to the stray field (7 mT) for a duration 1 s and then returned to the magnet. After another 10 minutes the process was repeated once more. An 13C-NMR spectrum of the solid sample was recorded and the signal was found to be in accordance with thermal equilibrium at 6.56 T and 2.5 K. T1-values for the ^{13}C atom in 2-^{13}C-2,2-bis (trideuteromethyl)-1,1,3,3-tetradeuteropropane-1,3-diol

H_2O, air saturated, 37° C., 7 T	133 s
H_2O, degassed, 37° C., 7 T	157 s
Human bloodplasma, 37° C., 7 T	96 s
Solid, 20° C., 7 T	237 s
Solid, 2.5 K, 7 T	45 hours

EXAMPLES 8–9

Solution Experiments

EXAMPLE 8

A sample of solid 1-^{13}C-2,2,2',2',2",2"-hexadeuterotris (hydroxymethyl)nitromethane (20 mg) was subjected to the above-mentioned pumping procedure (see Examples 5–7) and then in less than 1 second moved to a holding field of 0.4 T where also a sample of deuterium oxide (3 ml) at a temperature of 40° C., stirred by nitrogen bubbling, was kept. The solid was added to the liquid and a clear solution was obtained in less than 1 S. This solution was pipetted over to a 5 mm standard NMR-sample tube and moved to a nearby NMR-spectrometer while kept in a holding field of 10 mT. The sample was inserted into the spectrometer and a ^{13}C-spectrum was recorded. The whole process of moving the sample out of the cryomagnet, dissolution, sample preparation, transport and spectroscopy took 35 s. The intensity of the ^{13}C-signal was compared to the intensity after the sample had reached thermal equilibrium at 40° C. and 7 T. An enhancement factor of 12 was found.

EXAMPLE 9

A sample of solid 2-^{13}C-2,2-bis(trideuteromethyl)-1,1,3,3-tetradeuteropropane-1,3-diol (20 mg) was subjected to the above-mentioned pumping procedure (see Examples 5–7) and then in less than 1 second moved to a holding field of 0.4 T where also a sample of deuterium oxide (3 ml) at a temperature of 40° C., stirred by nitrogen bubbling, was kept. The solid was added to the liquid and a clear solution was obtained in less than 1 S. This solution was pipetted over to a 5 mm standard NMR-sample tube and moved to a nearby NMR-spectrometer while kept in a holding field of 10 mT. The sample was inserted into the spectrometer and a ^{13}C-spectrum was recorded. The whole process of moving the sample out of the cryomagnet, dissolution, sample preparation, transport and spectroscopy took 35 s. The intensity of the ^{13}C-signal was compared to the intensity after the sample had reached thermal equilibrium at 40° C. and 7 T. An enhancement factor of 21 was found.

What is claimed is:

1. A method of magnetic resonance (MR) investigation of a sample, said method comprising:

(i) producing a hyperpolarised solution of a high T_1 agent by dissolving in a physiologically tolerable solvent a hyperpolarised solid sample of said high T_1 agent;

(ii) where the hyperpolarisation of the solid sample of said high T_1 agent in step (i) is effected by means of a polarising agent, optionally separating the whole, substantially the whole, or a portion of said polarising agent from said high T_1 agent;

(iii) administering said hyperpolarised solution to said sample;

(iv) exposing said sample to radiation of a frequency selected to excite nuclear spin transitions in an MR imaging nuclei of the high T_1 agent;

(v) detecting magnetic resonance signals from said sample; and

(vi) optionally, generating an image, dynamic flow data, diffusion data, perfusion data, physiological data or metabolic data from said detected signals,

wherein said high T_1 agent in said hyperpolarised solution has a T_1 value (at a field strength in the range 0.01–5 T and a temperature in the range 20–40° C.) of at least 5 seconds and wherein said high T_1 agent is ^{13}C enriched at one or more carbonyl or quaternary carbon positions.

2. A method as claimed in claim 1 wherein said high T_1 agent or the hyperpolarised solution thus formed is transported in a magnetic field and at low temperature between steps (i) and (ii) and such that said agent or said solution retains its polarisation during said transportation.

3. A method as claimed in claim 2 wherein the magnetic field during said transportation is greater than 10 mT.

4. A method as claimed in claim 2 wherein the magnetic field during said transportation is greater than 1 T.

5. A method as claimed in claim 2 wherein the temperature during said transportation is lower than 80 K.

6. A method as claimed in claim 1 wherein during said dissolution step (i), the high T_1 agent is soluble in said physiologically tolerable solvent to a concentration of at least 1 mM at a rate of 1 mM/3 T_1.

7. A method as claimed in claim 6 wherein said high T_1 agent is soluble in said physiologically tolerable solvent to a concentration of at least 1 mM at a rate of 1 mM/T_1.

8. A method as claimed in claim 1 wherein a magnetic field is present during the dissolution stage and wherein said magnetic field is greater than 10 mT.

9. A method as claimed in claim 1 wherein step (i) comprises polarising a solid high T_1 agent by irradiating a polarising agent whereby to cause dynamic nuclear polarisation.

10. A method as claimed in claim 1 wherein said high T_1 agent has a T_1 value (at a field strength of 0.01–5 T and a temperature in the range 20–40° C.) of at least 10 secs.

11. A method as claimed in claim 1 wherein said high T_1 agent exhibits a chemical shift of more than 2 ppm per 2 pH units.

12. A method as claimed in claim 11 wherein said chemical shift is per Kelvin.

13. A method as claimed in claim 11 wherein said chemical shift is upon being metabolised.

14. A method of magnetic resonance (MR) investigation of a sample, said method comprising:

(i) producing a hyperpolarised solution of a high T_1 agent by dissolving in a physiologically tolerable solvent a hyperpolarised solid sample of said high T_1 agent;

(ii) where the hyperpolarisation of the solid sample of said high T_1 agent in step (i) is effected by means of a polarising agent, separating the whole, substantially the whole, or a portion of said polarising agent from said high T_1 agent;

(iii) administering said hyperpolarised solution to said sample;

(iv) exposing said sample to radiation of a frequency selected to excite nuclear spin transitions in an MR imaging nuclei of the high T_1 agent;

(v) detecting magnetic resonance signals from said sample; and

(vi) optionally, generating an image, dynamic flow data, diffusion data, perfusion data, physiological data or metabolic data from said detected signals,

wherein said high T_1 agent in said hyperpolarised solution has a T_1 value (at a field strength in the range 0.01–5 T and a temperature in the range 20–40° C.) of at least 5 seconds,

and wherein step i) comprises polarising a hyperpolarisable gas before, during or after introducing said high T_1 agent thereto whereby to cause nuclear polarisation of said high T_1 agent.

15. A method as claimed in claim 14 wherein said high T_1 agent has a T_1 value (at a field strength of 0.01–5T and a temperature in the range 20–40° C.) of at least 10 secs.

16. A method as claimed in claim 14 wherein said high T_1 agent contains ^{13}C, ^{15}N, ^{29}Si, ^{31}P, ^{1}H or ^{19}F nuclei.

17. A method as claimed in claim 16 wherein said high T_1 agent is ^{13}C enriched at one or more carbonyl or quaternary carbon positions.

18. A method as claimed in claim 14 wherein said high T_1 agent exhibits a chemical shift of more than 2 ppm per 2 pH units.

19. A method as claimed in claim 18 wherein said chemical shift is per Kelvin.

20. A method as claimed in claim 18 wherein said chemical shift is upon being metabolised.

21. A method as claimed in claim 14 wherein said high T_1 agent has ^{13}C or ^{15}N or ^{29}Si at one particular position in its molecular structure in an amount above 5%.

22. A method as claimed in claim 14 wherein said high T_1 agent has ^{13}C or ^{15}N or ^{29}Si at one particular position in its molecular structure in an amount above 99%.

23. A method as claimed in claim 14 wherein said hyperpolarisable gas is a noble gas.

24. A method as claimed in claim 14 wherein said hyperpolarisable gas is a mixture of two or more gases.

25. A method of magnetic resonance (MR) investigation of a sample, said method comprising:

(i) producing a hyperpolarised solution of a high T_1 agent by dissolving in a physiologically tolerable solvent a hyperpolarised solid sample of said high T_1 agent;

(ii) where the hyperpolarisation of the solid sample of said high T_1 agent in step (i) is effected by means of a polarising agent, optionally separating the whole, substantially the whole, or a portion of said polarising agent from said high T_1 agent;

(iii) administering said hyperpolarised solution to said sample;

(iv) exposing said sample to radiation of a frequency selected to excite nuclear spin transitions in an MR imaging nuclei of the high T_1 agent;

(v) detecting magnetic resonance signals from said sample; and

(vi) optionally, generating an image, dynamic flow data, diffusion data, perfusion data, physiological data or metabolic data from said detected signals,

wherein said high T_1 agent in said hyperpolarised solution has a T_1 value (at a field strength in the range 0.01–5 T and a temperature in the range 20–40° C.) of at least 5 seconds,

and wherein step i) comprises the polarisation stages of:

a) subjecting said high T_1 agent to a high magnetic field at low temperature;

b) exposing the agent to a T_1 shortening effect in order to attain thermodynamic equilibrium at said low temperature.

26. A method as claimed in claim **25** wherein said magnetic field of stage (a) of step (i) is greater than 1 T.

27. A method as claimed in claim **25** wherein said low temperature of stage (a) of step (i) is 100 K or less.

28. A method as claimed in claim **25** wherein said T_1 shortening effect is provided by exposure to a variable magnetic field gradient.

29. A method as claimed in claim **25** wherein said T_1 shortening effect is provided by field cycling to a field allowing cross polarisation.

30. A method as claimed in claim **25** wherein said T_1 shortening effect is provided by gradually increasing the magnetic field at such a rate that the increase in polarisation of the high T_1 agent is maximised.

31. A method as claimed in claim **25** wherein said T_1 shortening effect is provided by gradually decreasing the temperature at such a rate that the increase in polarisation of the high T_1 agent is maximised.

32. A method as claimed in claim **25** wherein said T_1 shortening effect is provided by adding a material with unpaired electrons during stage (a) of step (i) when the agent is exposed to low temperature.

33. A method of magnetic resonance MR investigation of a sample, said method comprising:

i) producing solid hyperpolarised ^{129}Xe by irradiating a polarising agent whereby to cause dynamic nuclear polarisation;

ii) dissolving said solid hyperpolarised ^{129}Xe in a physiologically tolerable solvent to form a solution;

iii) administering said solution of said hyperpolarised ^{129}Xe in a physiologically tolerable solvent to said sample; and

iv) detecting magnetic resonance signals from said sample.

34. A method as claimed in claim **25** wherein said high T_1 agent contains ^{13}C, ^{15}N, ^{29}Si, ^{31}P, ^{1}H or ^{19}F nuclei.

* * * * *

US006200658B1

(12) **United States Patent**
Walther et al.

(10) **Patent No.: US 6,200,658 B1**
(45) **Date of Patent: Mar. 13, 2001**

Please scan

(54) **METHOD OF MAKING A HOLLOW, INTERIORLY COATED GLASS BODY AND A GLASS TUBE AS A SEMI-FINISHED PRODUCT FOR FORMING THE GLASS BODY**

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(52) **U.S. Cl.** **428/34.4**; 428/34.5; 428/34.6; 138/145; 138/146

(58) **Field of Search** 428/34.4, 34.5, 428/34.6; 135/145, 146

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,314,772		4/1967	Poole et al. .
4,882,209	*	11/1989	Maruyama .
5,154,943		10/1992	Etzkorn et al. .

FOREIGN PATENT DOCUMENTS

39 24 830 A1	2/1991	(DE) .
40 08 405 C1	7/1991	(DE) .
94 04 753 U	7/1994	(DE) .
44 38 359 A1	5/1996	(DE) .
296 09 958 U	10/1996	(DE) .
196 29 877 C1	3/1997	(DE) .

OTHER PUBLICATIONS

"Entalkalisierung Von Na20–CAO . . . ", von Schaëfer et al, Glastechn. 54(1981), Nr. 8, pp. 247–256. (No month).

"Pachaging of Sensitive Parental Drugs in Glass Containers . . . " by M. Walther, Pharmaceutical Technology Europe, May 1996, vol. 8, Nr. 5., pp. 22–27.

* cited by examiner

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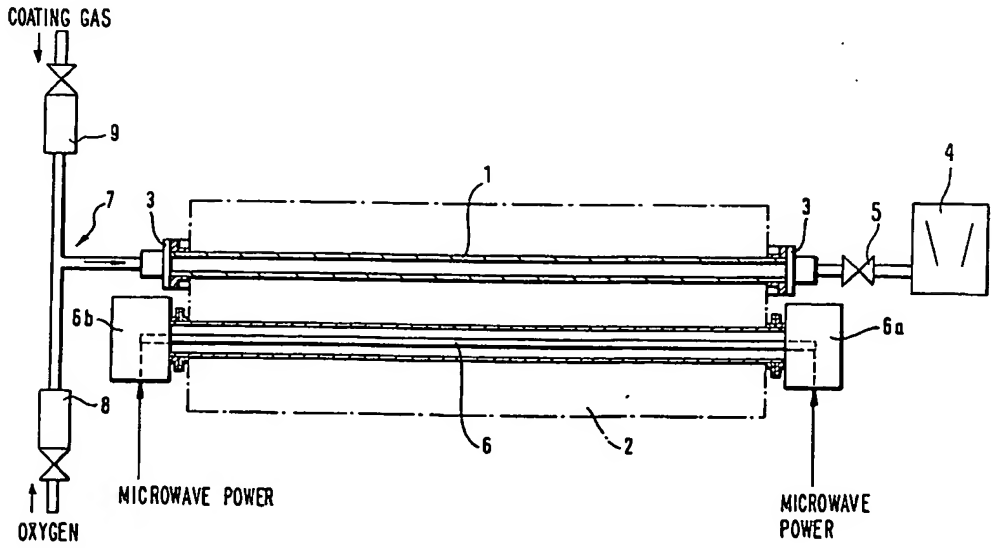
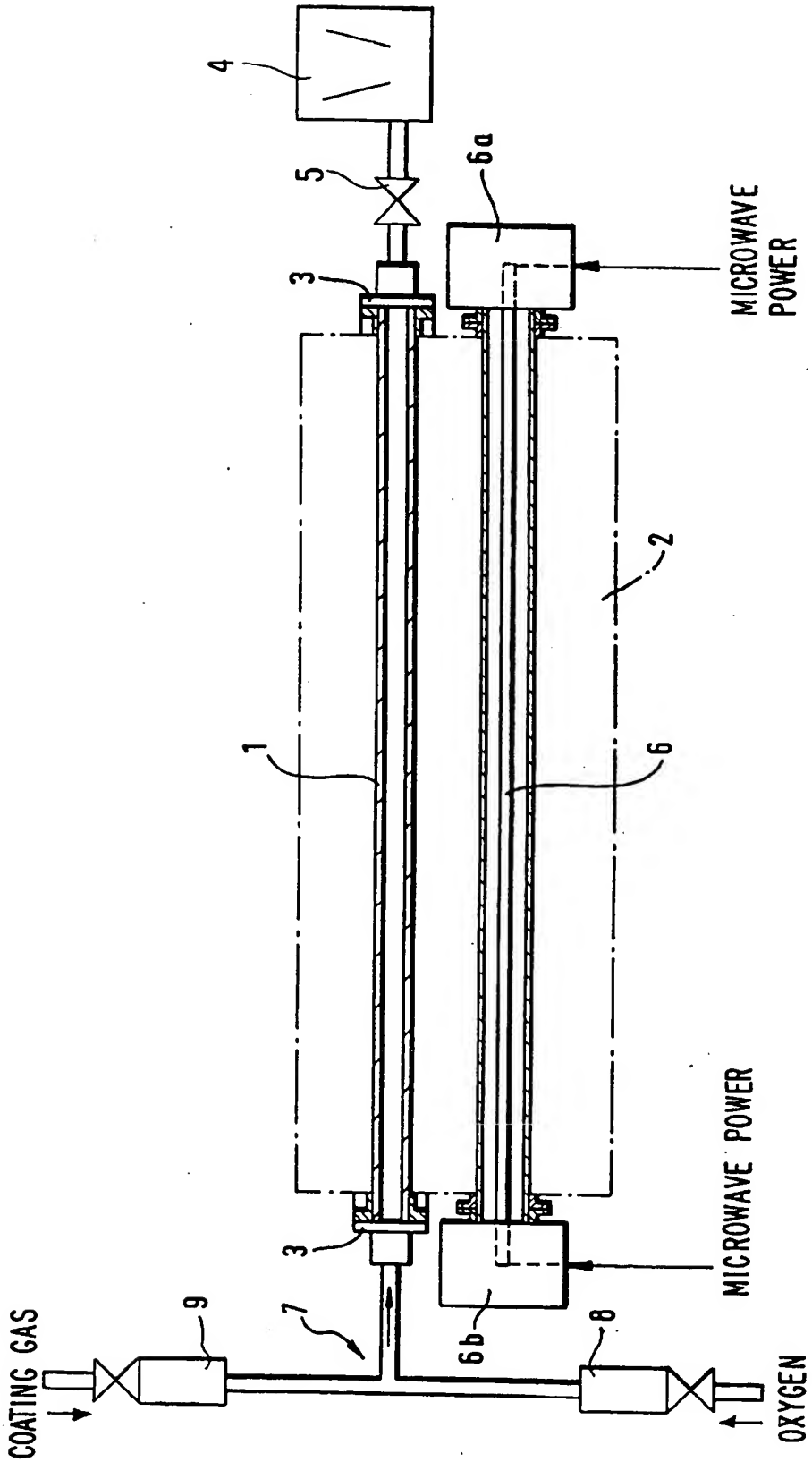
(57) **ABSTRACT**

Numerous applications for hollow glass bodies made from low melting glass material require an increase in the chemical resistance of the interior surface of the glass body. In order to avoid a disadvantageous de-alkalizing process the hollow glass body must be provided with an interior coating in a comparatively expensive prior art process. In an improved process according to the invention a glass tube acting as a semifinished product from which the hollow glass body is made is provided with an interior coating of oxide material, preferably SiO_2, Al_2O_3, TiO_2 or mixtures thereof, having a predetermined coating thickness according to the required chemical resistance or working conditions for forming the glass body and then the hollow glass body is made from the glass tube. The coating is advantageously provided by means of a PICVD process.

9 Claims, 1 Drawing Sheet




4
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COATING GAS
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METHOD OF MAKING A HOLLOW, INTERIORLY COATED GLASS BODY AND A GLASS TUBE AS A SEMI-FINISHED PRODUCT FOR FORMING THE GLASS BODY

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a method of making a hollow, interiorly coated glass body from a glass tube made of a low melting glass material and acting as semifinished product or intermediate product.

The invention also relates to a glass tube made from low melting glass material and acting as a semifinished product for forming a hollow glass body with an interior coating having a high chemical resistance or inertness.

2. Prior Art

Low melting glass materials, such as borosilicate glasses or calcium, sodium glasses, corrode in a known manner on contact with water or other liquids. Particularly water withdraws sodium ions from glass.

Thus it is necessary for numerous applications to increase the chemical resistance of the glass bodies, which are formed from this type of low melting glass, especially hollow glass bodies formed from glass tubes.

Hollow glass bodies, which require an increased chemical resistance for the interior surface, are, for example, those used

for chemical plant structures,

for flow meters for chemically reactive media,

for analytical purposes (e.g. burette tubes, titration cylinders, etc.),

for reagent glasses for special purposes,

for sheathing of measuring electrodes in reactive media,

for illumination purposes, e.g. halogen lamps,

for discharge lamps,

for components used for biotechnology reactors, and

as containers for medicinal purposes (e.g. ampoules, bottles, injector devices, cylindrical ampoules, etc.).

The latter mentioned applications are of special significance.

It is indeed known to make glass tubes from silica glass (quartz glass, SiO_2 glass) as a semifinished product for forming hollow glass bodies, which have a very high chemical resistance. Those glass tubes are however very expensive because of the high melting point of the SiO_2 glass. Furthermore they can only be made with limited optical quality and are less suitable for mass production. These tubes may be formed with only very special apparatus since, on the one hand, their forming temperatures are very high and, on the other hand, the temperature interval in which their formation is possible is very small.

Semifinished glass tubes made from silica glass thus may not be of sufficient quality and are uneconomical for mass applications.

Predominantly low melting glasses, e.g. borosilicate glasses or calcium-sodium glasses, are used for large-scale glass products. These may advantageously be formed as tubes economically.

For example these glasses include the following: Duran®-borosilicate glass (Schott Glas), Fiolax®klar(Schott glass, Fiolax®braun(Schott Glas) and Kimble N 51 A (Fa. Kimble).

The compositions of these glasses made in the form of glass tubing are tabulated in the following Table I.

TABLE I

GLASS COMPOSITIONS IN % by WEIGHT*

GLASS	SiO_2	B_2O_3	Al_2O_3	Na_2O	K_2O	MgO	CaO	BaO
1	69	1.0	4	12.5	3.5	2.5	5	2
2	69	1.0	4	12.5	3.5	2.5	5	2
3	69	1.0	4	12.5	3.5	2.5	5	2
4	70	1.0	4	12.5	3.5	2.5	5	2
5	69	1.0	4	12.5	3.5	2.5	5	2
6	69	1.0	4	12.5	3.5	2.5	5	2
7	75	11	5	7			1.5	0.5
8	75	11	5	7			1.5	0.5
9	80	13	2.5	3.5	0.5			
10	70.8	8	5.5	7	1.5		1	2
11	70.8	8	5.5	7	1.5		0.5	2
12	72.8	11	7	7	1		1	
13	73.3	10	6	6	3		0.5	
14	74.3	10	6	8	1			

*balance to 100% consists of other elements (for No. 10 and No. 11 Fe_2O_3 and TiO_2 which together are 3.5%)

It is known to increase the chemical resistance of these glass tubes made from low melting glass by a method in which the glass surface is chemically leached out. A sutable reactive gas (SO_2, $(NH_4)_2SO_4$ or HCl) is conducted through the still warm glass tube, which leads to a surface reaction and a reduction in the alkali content at the surface.

This type of dealkalizing process is, e.g., described in H. A. Schaeffer, et al, Glastechn. Ber. 54, Nr. 8. pp. 247 to 256. The disadvantage of this process is that predominantly toxic gasses are used, whereby the glass surface can contain traces of these reactive reaction gases after this chemical treatment and the glass surface structure is damaged which leads to an increased surface area and to an increase in reactive sites on the surface. Furthermore the use of these reactive gases is undesirable from an environmental standpoint and due to worker safety consideration. With many of the suggested gases corrosive by-products arise, which react strongly with metal apparatus parts. Furthermore particles can be released from the porous damaged surfaces during shaping or forming of this type of leached out glass tube. Also a washing process for removal of reaction products is necessary prior to use of the leached out glass tube. This washing process necessitates a drying and disposal of reaction products, i.e. the costs increase for making the semifinished glass tubes.

An additional process for dealkalizing low melting glass by fluorination by means of fluoro-acids, which has the same main disadvantages as the above-described process, is described in U.S. Pat. No. 3,314,772.

In order to avoid the disadvantages of dealkalizing process it is also known to provide a tubular glass container from low melting glass material, which operates as a packaging device for pharmaceutical materials, having a silicon dioxide (SiO_2) layer on its interior surface, which has the same inertness as a quartz glass surface (M. Walther, "Packaging of sensitive perenteral drugs in glass containers with a quartz-like surface", in Pharmaceutical Technology Europe, May, 1996, Vol. 8, Nr. 5, pp. 22 to 27.

The coating of the interior surface of the formed glass body occurs by chemical deposition of an oxide coating from the gas phase, especially by means of a vacuum-assisted plasma CVD process (PECVD=plasma enchanced chemical vapor deposition), in particular by means of a pulsed plasma process (PICVD=plasma impulse chemical vapor deposition).

This PECVD or PICVD method for coating of an interior of a hollow body, especially made from plastic, is known from German Patent Documents DE 196 29 877 and DE-Z

"Multilayer Barrier Coating System produced by Plasma-impulse Chemical Vapor Deposition(PRCVD)" by M. Walther, M. Hemming, M. Spallek, in "Surface and Coatings Technology" 80, pp. 200 to 205 (1966).

In the known case (DE 296 09 958 U1) the finished containers, i.e. the glass bodies themselves, are interiorly coated. Because of that each glass container, must be subjected to an expensive coating process, adapted to its form.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a simple and economical method of making a hollow glass body made from a low melting glass material.

It is another object of the present invention to provide a semifinished glass tube for making the hollow, interiorly coated glass body of the invention.

These objects and others which will be made more apparent hereinafter are attain in a process of the above-described type for making a hollow, interiorly coated glass body from a glass tube made of low melting glass material and acting as a semifinished product or intermediate.

According to the invention this process includes the steps of:

coating the interior surface of the semifinished glass tube with an oxide material to form an interior coating having a coating thickness which is adapted to the subsequent shaping or working conditions required for making the glass body and the chemical resistance requirements of the glass body, and

making the glass body from the interiorly coated semifinished glass tube.

The glass tube according to the invention acting as the semifinished product or intermediate for making the glass body has an interior surface provided with a coating of oxide material whose coating thickness adapted to the subsequent shaping or working conditions required for making the glass body and the chemical resistance requirements of the glass body.

Glass tubes are prepared with the methods of the invention whose chemical resistance is largely maintained after a working or shaping process. These working or shaping processes can include constrictions, melting and shaping at the ends of the glass tubes, e.g. in order to be able to join them together, to connect them, to close them, etc.

The invention not only concerns the manufacture of hollow glass bodies with a high degree of shaping, i.e. the forming of such glass bodies, but also those glass bodies with a comparatively reduced degree of shaping or working, e.g. cylindrical bodies, which are made from semifinished articles by hot forming or cold forming, e.g. a drawing process, and which must be still worked only on their opposite ends. These glass bodies include, for example, an injector cylinder, e.g. according to German Patent Document DE 39 24 830 A1 or a reagent container according to German Patent Document DE 94 04 753.7 U1 or an injector cylinder open on both ends, which is closed by two stoppers and on which a needle attachment can be provided.

Because of the invention it is also possible to prepare glass tubes with increased interior chemical resistance so that the predominant part of the surface of the entire system is provided with a high chemical resistance after a possible shaping process, while a comparatively smaller area portion is left with a lesser chemical resistance. Exemplary applications include: glass tubes which are used in biotechnology and are used with media which is absorbed in standard glass surfaces, containers for medical purposes in which the total ion leach out from the container plays an important role, (e.g. for dispensing alkali and other metal ions).

When comparatively long glass tubes used as intermediate products for making the glass bodies are coated in a working process, the interiorly coated glass bodies can be made in a simple and economical manner, since the coating can be predominantly maintained after shaping. A semifinished product (or semiproduct) is a half-finished product, an article that is an intermediate between the raw material and the finished product, which however is obtained by subsequently performing different finishing steps.

Methods for interiorly coating glass tubes are known in themselves. These glass tubes are used, e.g., as pre-forms for optical fibers for transmitting light and information. Two optically different types of glass are made in the interior of a tube, which however in order to be able to be drawn out as a fiber must have very similar thermal properties (softening and shaping temperatures) and expansion coefficients.

In the known cases however glass tubes made from low melting glass material cannot act as semifinished products for forming or shaping of hollow glass bodies having an interior coating made of oxide material for increasing the chemical resistance of the glass interior surface.

The coating thickness of the oxide material is adjusted to the working or shaping conditions and the chemical resistance requirements. Both these requirements interfere with each other to some extent, since a thick coating guarantees a great chemical resistance, but impairs or prevents satisfactory working or shaping. A definite specific concrete statement of the required thickness range for the coating is not possible, but instead the coating thickness must be adapted to the particular shaping or working process being performed and to the chemical resistance requirements.

A typical coating thickness range is according to a preferred embodiment of the invention in a range of from 1 nm to 500 nm or from about 1 nm to 500 nm. The coating thickness also depends on the material selected for the coating.

According to a preferred embodiment of the invention the following oxides may be used, among others, as coating materials: SiO_2, Al_2O_3, TiO_2 or mixtures thereof.

The following methods are especially preferred for coating the interior surface of semifinished glass pipe.

Methods for coating from the liquid phase (Sol Gel coating), for example, are described in H. Bach, D. Krause, "Thin Films on Glass", Springer Verlag, Berlin (1997).

Methods are known for precipitation from supersaturated solutions.

Sputtering methods, even when their use for pipe-like substrates is complicated, can be used, since sputtering process are direct processes.

Advantageously CVD processes (CVD=chemical vapor deposition) can be used for making of the semifinished glass tube. The coating is produced at elevated temperatures (i.e. higher than room temperature) in so-called thermal CVD methods. These methods can be used directly during the manufacture of the glass tube after the known drawing process. For this purpose the coating gas is used as supporting air/blowing air. The coating gas decomposes in a predetermined temperature range in the glass tube and forms a coating on its interior tube surface. A suitable similar method can of course be employed which is independent of the manufacture of the glass tube however re-heating of the glass tube is then required. The subsequent heating can occur

by different methods, e.g. direct heating, heating with a laser and so forth. It is also possible to reduce the coating temperature when light radiation is used for activation/production of the active coating conditions.

Advantageously the deposition of the oxide coating material can occur from the gas phase, from the coating gas, by means of a vacuum-assisted plasma CVD method, the so-called PECVD processes (plasma enhanced chemical vapor deposition). The PECVD process is described in various references. Diverse embodiments are used with different energy input in the low frequency range (e.g. 40 kHz), in the middle frequency range (e.g. 13.56 MHz) up to the microwave range (2.45 GHz and above). Examples are found in G. Janzen, "Plasma-technik(Plasma Engineering)", Hutig-Verlag, Heidelbery, 1992.

In a preferred embodiment which is especially advantageous a modified PECVD method, the so-called PICVD process (plasma-impulse-CVD process) is used, which provides a high uniformity for large-scale coated substrates. The PICVD technology is known in the patent literature from German Patent Document DE 40 08 405 C1 and from U.S. Pat. No. 5,154,943 and for example used for producing barrier layers on plastic containers (German Patent Document DE 44 38 359 A1). This technology uses pulsed plasmas for deposition of coatings from the respective coating gases.

BRIEF DESCRIPTION OF THE DRAWING

The objects, features and advantages of the invention will now be illustrated in more detail with the aid of the following description of the preferred embodiments, with reference to the accompanying sole figures which is a cross-sectional view through an apparatus for interior coating of a glass tube according to the method of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The coating apparatus shown in the figure operates according to the PICVD process. A glass tube section **1** made from low melting glass material, such as borosilicate or calcium-sodium glass, which is to be coated inside and which acts as a semifinished product or intermediate for making the interiorly coated, hollow glass body, is held in a container **2** in a vacuum-tight manner by means of the seals **3**.

The glass tube section **1** has a length of 1500 mm and an interior diameter of 12 mm in the embodiment shown in the drawing.

The length of the glass tube section to be coated conforms to the dimensions of the available coating apparatus.

The interior of the glass tube section **1** is connected to one end of a vacuum system comprising a pump **4** and a valve **5**.

A microwave supply device **6** comprising electrodes (antennas) passes through the container **2**. Microwave radiation is coupled impulse-wise into both ends of the microwave supply device **6** by means of suitable microwave blocks *6a*, *6b*. The duration of the microwave pulse is in a range of from 0.1 to 10 ms.

The interior of the glass tube is connected at its other end with a gas supply apparatus **7**. The gas, in which a plasma is ignited, typically oxygen, is conducted into the interior of the glass tube by means of this gas supply apparatus via a mass flow regulator **8**. Another gas, the reaction gas, required for forming the coating, is also conducted into the interior of the glass tube by means of this glass supply apparatus via another mass flow regulator **9**.

The reaction gas typically is a metal-organic reaction gas, such as siloxane, preferably hexamethyldisiloxane (HMDSO), tetramethyldisiloxane, titaniumtetraisopropoxide (TIPT) or silazane, from which the coating on the inside of the glass tube **1** is formed by selection of the suitable pulse duration. The pulse duration is an additional parameter, which also influences the composition of the deposited coating.

The coating process is controlled in a known manner by an unshown process controller.

First the entire tube system is evacuated and then the process pressure is controlled so that it is about 1 mbar. After that the oxygen is conducted into the system with flow of 135 standard cubic units. After 5 s 2.45 GHz microwave radiation at a power of 1 kW is input to both sides of the glass tube **1** by means of the electrodes of the microwave supply device. Because of that the plasma ignites inside the glass tube **1** and the glass tube is heated to a process temperature of 250° C. When this temperature is reached, a mass flow of 5 standard cubic units of reaction gas, preferably HMDSO, is supplied under control of the mass flow regulator **9**, so that a gas mixture of oxygen and HMDSO is found inside the glass tube **1**. Now a microwave power of 1.5 kW is coupled impulse-wise into the plasma inside the glass tube **1** by means of the electrodes **6**, whereby the molecules of the reaction gas are cracked. The cracking products produced diffuse to the closest surface,—here the glass tube to be coated—and in due course form the desired coating. In the interval between pulses until the following pulse is ignited, which is in a range of from 10– to 100 ms, the consumed reaction gases are removed from the vacuum chamber by means of the vacuum stages **4,5** in the same manner as a two cycle motor and replaced by fresh reaction gas and oxygen.

In this manner a coating with the thickness of 5 nm can be deposited in 2 s.

The properties of the coating substantially depend on the parameters "pulse duration" and "reaction gas concentration". Generally harder coatings are deposited at small concentrations and with long pulses, which cause a substantial increase in inertness. At high concentrations and with short pulses, softer layers are deposited.

Basically a multilayer coating can be produced. Furthermore as soon as a sufficient layer thickness is obtained for the first layer, the reaction gas required to produce it is replaced by a reaction gas for the second layer. To produce a non-discontinuous or non-sharp transition between both layers, a mixture of both reaction gases can be conducted into the apparatus for a predetermined time interval. For a uniform transition the proportion of the first reaction gas can be gradually reduce and at the same time the proportion of the second reaction gas can be continuously increased to its nominal value.

Additionally or instead of oxygen as the plasma gas or gas for producing the plasma, other gases for producing a plasma which are known, such as argon, helium, hydrogen or nitrogen. Other gases for producing the plasma are described, e.g., in the book, "Plasma-Technik(Plasma Enginering)", by Schade, Verlag Technik (Engineering Press), GmbH, Berlin, 1990.

EXAMPLE

Four samples with coating thicknesses of 0.5 nm, 1 nm, 5 nm and 50 nm are prepared by variation of the coating time

with the apparatus shown in the drawing. Ampoules are formed as glass bodies from the coated semifinished glass tubes. Both the unfinished glass tube samples and the finished ampoules, including an uncoated sample, were tested with the help of atomic absorption spectroscopy for limiting values according to ISO 4802, Part II, after autoclaving with steam.

The results for the Na leach out after one hour are shown in Table II hereinbelow, for the tubing in column 2 and for the ampoules in column 4.

Also the workability or formability of the ampoules from the crude or unfinished tube samples was evaluated qualitatively. These evaluations are also shown in Table II in column 3 for the respective coating thickness. The results show that the glass tube with a coating thickness of 50 nm is not workable or formable and the glass tube with a coating thickness of 5 nm has poor workability or formability and is unsatisfactory for making the ampoules.

TABLE II

MEASUREMENTS OF THE PROPERTIES AND WORKABILITY OF SEMIFINISHED GLASS TUBE SAMPLES AND AMPOULES MADE FROM THEM

COATING THICKNESS	NA LEACH OUT (TUBE), PPM	WORKABILITY (TO AMPOULES)	NA-LEACH OUT (10 ML), PPM
Uncoated	0.54 ppm	Good	0.96 ppm
0.5 nm	0.11 ppm	Good	0.21 ppm
1 nm	0.04 ppm	Good	0.12 ppm
5 nm	<0.01 ppm	Poor	0.30 ppm
50 nm	<0.01 ppm	Impossible	No measurement Possible

The results in Table II shows that the blocking action of the coating increases with increasing coating thickness, but at the same time ampoules cannot be made if the coating is too thick. An optimum coating thickness with minimum sodium leach out is expected between a coating thickness of 1 nm and 5 nm with this coating system.

The disclosure in German Patent Application 198 01 861.4-45 of Jan. 20, 1998 is incorporated here by reference. This German Patent Application describes the invention described hereinabove and claimed in the claims appended hereinbelow and provides the basis for a claim of priority for the instant invention under 35 U.S.C. 119.

While the invention has been illustrated and described as embodied in a method of making a hollow, interiorly coated glass body and glass tube as semifinished product for forming the glass body, it is not intended to be limited to the details shown, since various modifications and changes may be made without departing in any way from the spirit of the present invention.

Without further analysis, the foregoing will so fully reveal the gist of the present invention that others can, by applying current knowledge, readily adapt it for various applications without omitting features that, from the standpoint of prior art, fairly constitute essential characteristics of the generic or specific aspects of this invention.

What is claimed is new and is set forth in the following appended claims.

We claim:

1. A glass tube made from low melting glass material and acting as a semi-finished product or intermediate for making a hollow, interiorly coated glass body with an interior coating increasing a chemical resistance of said hollow, interiorly coated glass body, said glass body being formed by shaping or working, wherein said glass tube has an interior surface and a coating of oxide material on said interior surface, and said coating has a predetermined coating thickness, whereby required shaping or working conditions for making said glass body from said glass tube and chemical resistance requirements for said glass body are met.

2. The glass tube as defined in claim 1, wherein said coating thickness is in a range from 1 nm and 500 nm or from about 1 nm to 500 nm.

3. The glass tube as defined in claim 1, wherein said coating thickness is between about 1 to 5 nm.

4. The glass tube as defined in claim 1, wherein said oxide material comprises SiO_2, Al_2O_3, TiO_2 or mixtures thereof.

5. The glass tube as defined in claim 1, wherein said coating is provided on the interior surface of the glass tube by a method comprising a chemical vapor deposition process and including passing a mixture of a reaction gas and oxygen through the glass tube and forming a microwave discharge in the mixture in the glass tube, said reaction gas comprising hexamethyidisiloxane, tetramethyldisiloxane, titanium tetraisopropoxide or silazane.

6. A glass tube with an interior oxide coating, said glass tube with said interior oxide coating being made by a method comprising the steps of:

a) providing a glass tube consisting of a low melting glass material and having an interior surface; and

b) coating said interior surface of said glass tube provided in step a) with an oxide selected from the group consisting of SiO_2, Al_2O_3 and TiO_2 or with a mixture of at least two members selected from the group consisting of SiO_2, Al_2O_3 and TiO_2 to form the interior oxide coating with a thickness of from about 1 nm to 500 nm;

whereby said glass tube with said interior oxide coating is an intermediate product for making a hollow, interiorly coated glass body from said glass tube by shaping or working so that said hollow, interiorly coated glass body has an increased chemical resistance.

7. The glass tube as defined in claim 6, wherein said thickness is from about 1 nm to 5 nm.

8. The glass tube as defined in claim 6, wherein said method comprises chemical vapor deposition (CVD) of said interior oxide coating on said interior surface from a gas phase by vacuum-assisted plasma enhanced chemical vapor deposition or plasma impulse chemical vapor deposition.

9. The glass tube as defined in claim 6, wherein said method comprises depositing said oxide coating from a liquid phase according to a sol-gel process or from a supersatured solution.

* * * * *

US005545396A

United States Patent [19]

Albert et al.

[11] Patent Number: 5,545,396

[45] Date of Patent: Aug. 13, 1996

[54] MAGNETIC RESONANCE IMAGING USING HYPERPOLARIZED NOBLE GASES

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[73] Assignees: The Research Foundation of State University of New York, Albany, N.Y.; The Trustees of Princeton University Office of Research and Project Administration, Princeton, N.J.

[21] Appl. No.: 225,243

[22] Filed: Apr. 8, 1994

[51] Int. Cl.[6] A61B 5/055

[52] U.S. Cl. 424/93; 424/9.37; 534/7; 436/173

[58] Field of Search 424/9, 9.3, 9.37; 436/173; 534/7; 128/653.4, 654

PLEASE SCAN

[56] References Cited

U.S. PATENT DOCUMENTS

4,586,111	5/1986	Clark, Jr.	128/653.4
4,775,522	10/1988	Clark, Jr.	424/9
4,793,357	12/1988	Lindstrom	128/654
4,862,359	8/1989	Trivedi et al.	364/413.05
4,996,041	2/1991	Arai et al.	424/9

OTHER PUBLICATIONS

Albert et al. *Chem. Abstracts* 121:128933r (1994) from: *Nature* 370 (6486):199–201 (1994).

Pfeffer, M *Chem. Abstracts* 121:174319 (1994).

Burt, C. T., Moore, R. R., Roberts, M. F., and Brady, T. J., *Biochim. Biophys. Acta.*, 805:375 (1984).

Mason, J., in *Multinuclear NMR*, Mason, J., ed., pp. 606–607, Plenum Press, New York (1987).

Jameson, C. J., Jameson, A. K., and Hwang, J. K., *J. Chem. Phys.*, 89:4074 (1988).

Laloë, F., Nacher, P. J., Leduc, M., and Schearer, L. D., *AIP Conf. Proc.*#131 (Workshop on Polarized ^{3}He Beams and Targets) (1984).

Ernst et al., *Principles of Nuclear Magnetic Resonance in One and Two Dimensions* (1987). pp. 538–596.

Derek Shaw, et al., eds., *Biomedical Magnetic Resonance Imaging*(1988) Ch. 1 and 3. pp. 1–46.

Haase, A., Frahm, J., Matthaei, D., Hanicke, W., and Merboldt, K. D., *J. Magn. Reson.*, 67:258–266 (1986).

Norberg, R. E., in *Rare Gas Solids*, eds. Hohler, G., Springer–Verlag, New York (1984). pp. 59–95.

Kaplan, H. M., Brewer, N. R., and Blair, W. H., in *The Mouse in Biomedical Research*, eds. Foster, H. L., Small, J. D., and Fox, J. G., pp. 250–278, Academic Press, New York (1983).

Wyrwicz, A.M., Schofield, J. C., Tillman, P. C., Gordon, R. E., and Martin, P. A., *Science*, 222:428 (1983).

Miller, K. W., Reo, N. V., Uiterkamp, A. J. M. S., Stengle, D. P., Stengle, T. R., and Williamson, K. L., *Proc. Nat'l Acad. Sci. USA*, 78:4946 (1981).

Evers, A. S., Berkowitz, B. A., and d'Avignon, D. A., *Nature*, 328:157 (1987).

Wyrwicz, A. M., Li, Y.–E., and Schofield, J. C., *FEBS Lett.*, 162:334 (1983).

Burt, C. T., Moore, R. R., and Roberts, M. F., *J. Magn. Reson.*, 53:163 (1983).

Lockhart, S. H., Cohen, Y., Yasuda, N., Kim, F., Litt, L., Eger, E. I., Chang, L.–H., and James, T., *Anesthesiology*, 73:455 (1990).

Barany, M., Spigos, D. G., Mok, E., Venkatasubramanian, P. N., Wilbur, A. C., and Langer, B. G., *Magn. Reson. Imaging*, 5:393 (1987).

Fullerton, G. D., and Cameron, I. L., Chapter 3 in *Biomedical Magnetic Resonance Imaging: Principles, Methodology, and Applications*, Wehrli, F. W., Shaw, D. S., and Kneeland, J. B., eds., pp. 115–155, VCH Publishers, New York (1988).

Susskind, H., Atkins, H. L., Klopper, J. K., Ansari, A. N., Richards, P., and Farchild, R. G., *Prog. Nucl. Med.*, 5:144 (1978).

Susskind, H., Ellis, K. J., Atkins, H. L., Cohn, S. H., and Richards, P., *Prog. Nucl. Med.*, 5:13 1978).

Kendall, B. E., and Moseley, I. F., *J. Neuroradiology*, 8:3 (1981).

Imai, A., Meyer, J. S., Kobari, M., Ichijo, M., Shinohara, T., and Oravez, *Neuroradiology*, 30:463–472 (1988). (first page only).

Yonas, H., Sekhar, L., Johnson, D. W., and Gur, D., *Neurosurgery*, 24:368 (1989).

(List continued on next page.)

Primary Examiner—Gary E. Hollinden
Attorney, Agent, or Firm—Hoffmann & Baron

[57] ABSTRACT

A method of imaging a spatial distribution of a noble gas by nuclear magnetic resonance spectrometry includes detecting a spatial distribution of at least one noble gas by NMR spectrometry and generating a representation of said spatial distribution of the noble gas. The noble gas is selected from noble gas isotopes having nuclear spin, preferably Xenon-129 and/or Helium-3. The noble gas is at least thermally or equilibrium polarized and is preferably hyperpolarized, most preferably hyperpolarized by optical (laser) pumping in the presence of an alkali metal or by metastability exchange. The generation of the representation of the noble gas spatial distribution includes at least one dimension, preferably 2 or 3 dimensions of the spatial distribution. The noble gas may be imaged according to the invention in chemical or biological systems, preferably in a human or animal subject or organ system or tissue thereof. Also, apparatus for nuclear magnetic resonance imaging of the spatial distribution of at least one noble gas includes means for imaging a noble gas by NMR spectrometry and means for providing and/or storing imageable quantities of a noble gas, preferably hyperpolarized Xenon-129 and/or Helium-3. Also, a medical composition includes a medically acceptable bifunctional gas effective for in vivo anesthesiological and NMR imaging functions, including at least one noble gas, preferably hyperpolarized Xenon-129 and/or Helium-3.

46 Claims, 7 Drawing Sheets

OTHER PUBLICATIONS

Albert, M. S., Springer, C. S., Murphy, R., and Winshnia, A., *Abs., 11th Ann. Mtg. Soc. Magn. Reson. Med.*, 2104 (1992).
Albert, M. S., Springer, C. S., and Wishinia, A., *Abs., 11th* Ann. Mtg. Soc. Magn. Reson. Med., 4710 (1992).
Carver, T. R., *Science*, 141:599 (1963).
Happer, W., Miron, E., Schafer, S., Schreiber, D., van Wijngaarder, W. A., and Zeng, X., *Phys. Rev. A*, 29:3092 (1984).
Wagshul, M. E., and Chupp, T. E., *Phys. Rev. A*, 40:4447 (1989).
Wagshul, M. E., Thesis, Department of Physics, Harvard University (1991).
Grover, B. C., *Phys. Rev. Lett.*, 40:391 (1978).
Schaefer, S. R., Cates, G. D., Chien, T.-R., Gonatas, D., Happer, W., and Walker, T. G., *Phys. Rev. A*, 39:5613 (1989).
Schaefer, S. R., Cates, G. D., and Happer, W., *Phys. Rev. A*, 41:6063 (1990).
Schearer, L. D., in *Phys. Rev. Lett.*, 21:660 (1968).
Schearer, L. D., in *Phys. Rev.*, 188:505 (1969).
Schearer, L. D., in *Phys. Rev.*, 180:83 (1969).
Colegrove, F. D., Schearer, L. D., and Walters, G. K., *Phys. Rev.*, 132:2561 (1963).
Hadeishi, T., and Liu, C.-H., *Phys. Rev. Lett.*, 19:211 (1967).
Schearer, L. D., *Phys. Lett.*, 28A:660 (1969).
Cates, G. D., Benton, D. R., Gatzke, M., Happer, W., Hasson, K. C., and Newbury, N. R., *Phys. Rev. Lett.*, 65:2591 (1990).
Gatzke, M., Cates, G. D., Driehuys, B., Fox, D., Happer, W., and Saam, B., *Phys. Rev. Lett.*, 70:690 (1993).
Bhaskar, N. D., Happer, W., and McClelland, T., *Phys. Rev. Lett.*, 49:25 (1982).
Cates, G. D., Fitzgerald, R. J., Barton, A. S., Bogorad, P., Gatzke, M., Newbury, N. R., and Saam, B., *Phys. Rev. A.*, 45:4631 (1992).
Raftery, D., Long, H., Meersmann, T., Grandinetti, P. J., Reven, L., and Pines, A., *Phys. Rev. Lett.*, 66:584 (1991).
Raftery, D., Long, H., Reven, L., Tang, P., and Pines, A., *Chem. Phys. Lett.*, 191:385 (1992).
Long, H. W. Gaede, H. C., Shore, J., Reven, L., Bowers, C. R., Kritzenberger, J., Pietrass, T., and Pines, A., *J. Am. Chem. Soc.*, 115:8491 (1993).
Rinck et al., *An Introduction to Magnetic Resonance in Medicine*(1990).
Stark et al., eds., *Magnetic Resonance Imaging*, vol. 1, 2d ed. (1992) Chapters 1 and 2. pp. 3–65.
Hunt E. R., and Carr, H. Y., *Phys. Rev.*, 130:2302 (1963).
Tilton, Jr., R. F., and Kuntz, Jr., I. D., *Biochemistry*, 21:6850 (1982).
Diehl, P., and Jokisaari, J., *J. Magn. Reson.*, 88:669 (1990).
Cullen, S. C., and Gross, E. G., *Science*, 113:580 (1951).
Wilcock, R. J., Battino, R., Danforth, W. F., and Wilhelm, E., *J. Chem. Thermodyn.*, 10:317 (1978).
Blumgart, H. L., and Weiss, S., *J. Clin. Invest.*, 4:339–425 (1927).
Pollack, G. L., Himm, J. F., and Enyeart, J. J., *J. Chem. Phys.*, 81:3239 (1984).
Wishnia, A., *Biochemistry*, 8:5064 (1969).
Bouchiat, M. A., Carver, T. R., and Varnum, C. M., *Phys. Rev. Lett.*, 5:373 (1960).
Zeng, X., Wu, Z., Call, T., Miron, E., Schreiber, D., and Happer, W., *Phys. Rev. A*, 31:260 (1985).
Schoenborn, B. P., *Nature*, 208:760 (1965).
Moschos, A., and Reisse, J., *J. Mag. Reson.*, 95:603 (1991).
Manabe, A., Miyazaki, T., and Toyoshima, H., *Magn. Reson. in Med.*, 5:492–501 (1987).
Look, D. C., and Locker, D. R., *Rev. Sci. Instrum.*, 41:250–251 (1970).
Yen, W. M., and Norberg, R. E., *Phys. Rev.*, 131:269 (1963).
Knudsen, G. M., Pettigrew, K. D., Patlak, C. S., and Paulson, O. B., *Am. J. Physiol.*, 266:H987–H999 (1994).
Kanal, E., and Wehrli, F. W., Chapter 2 in *Biomedical Magnetic Imaging*, Wehril, F. W., Shaw, D., and Kneeland, J. B., eds., pp. 47–112, VCH Publishers, New York (1988).
Pollack, G. L., and Himm, J. F., *J. Chem. Phys.*, 77:3221–3229 (1982).
Robillard, Jr., K. A., and Wishnia, A., *Biochemistry*, 11:3835–3840 (1972).

FIG-1a

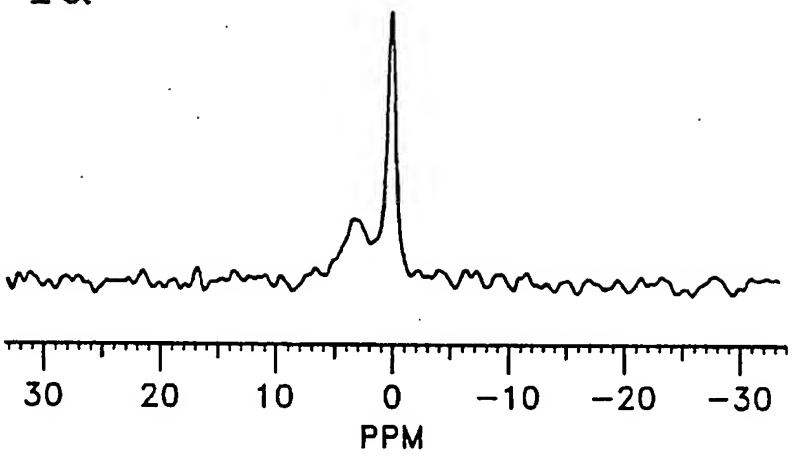


FIG-1b

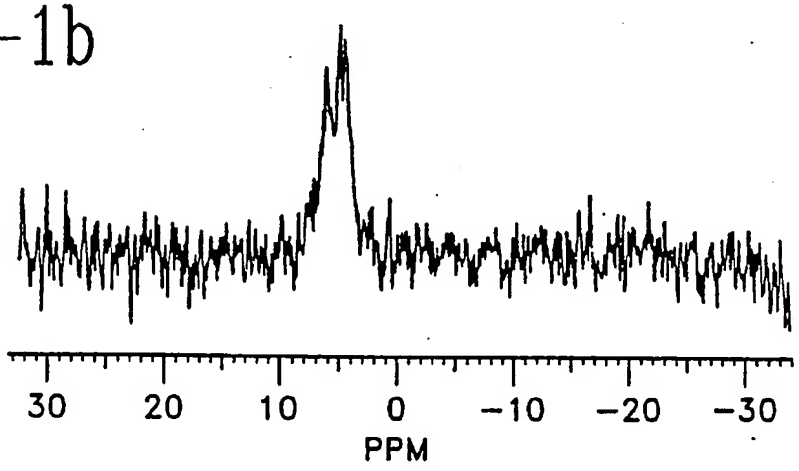


FIG-1c

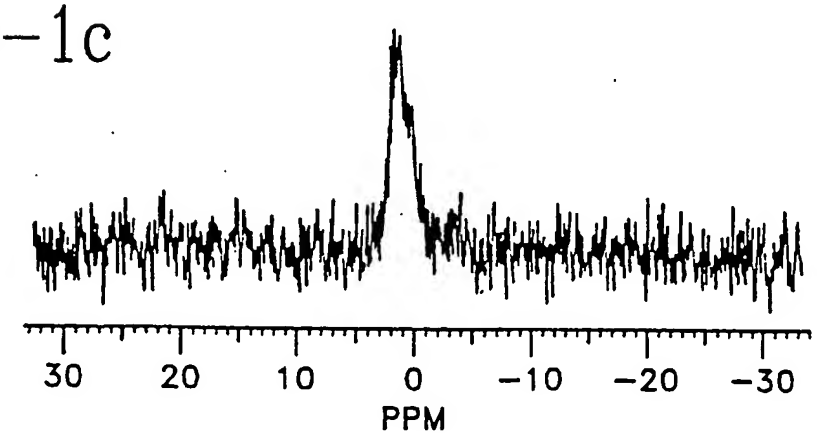


FIG-2a

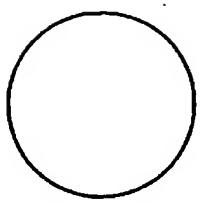
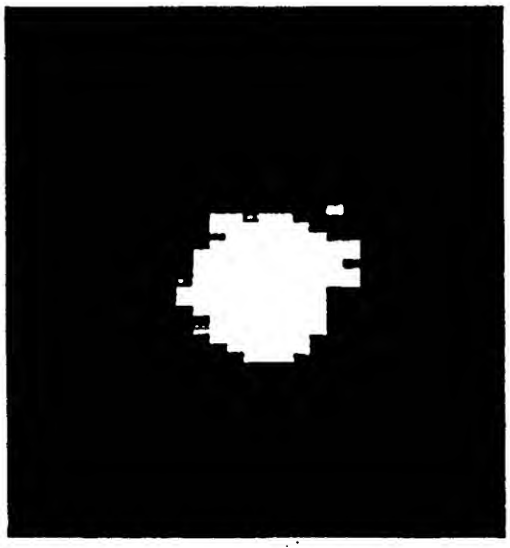
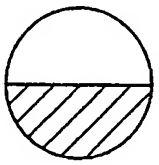


FIG-2b



FIG–3a



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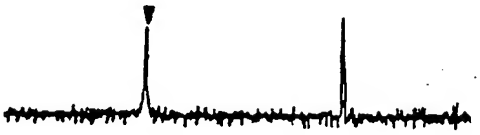

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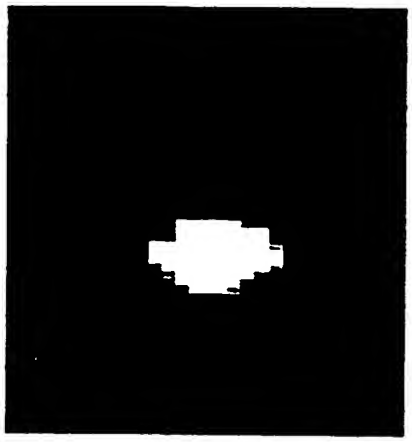


FIG–3d

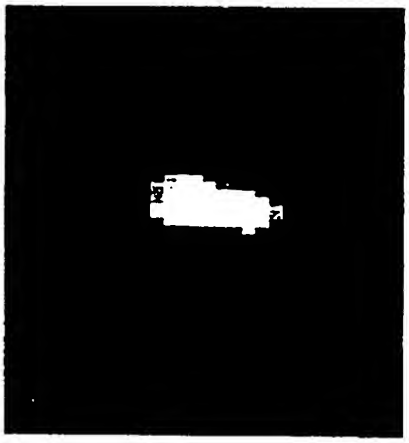


FIG-4a

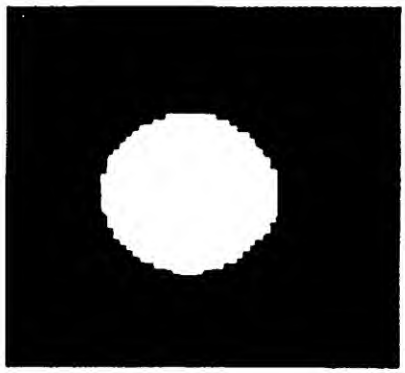


FIG-4b

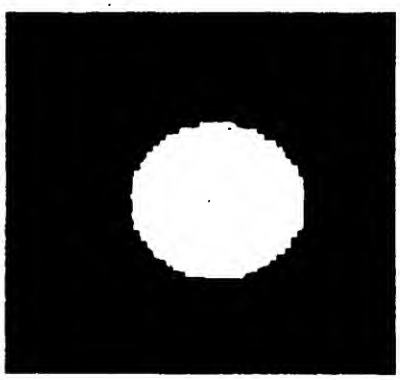


FIG-4c

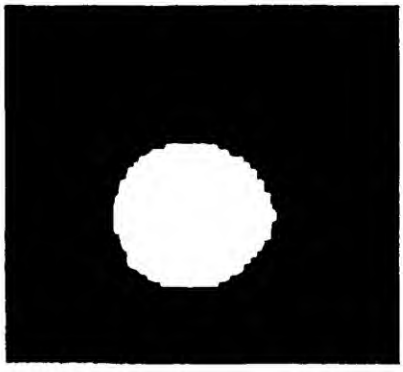


FIG-4d

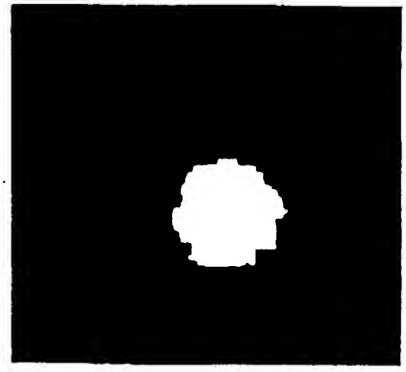


FIG-5a

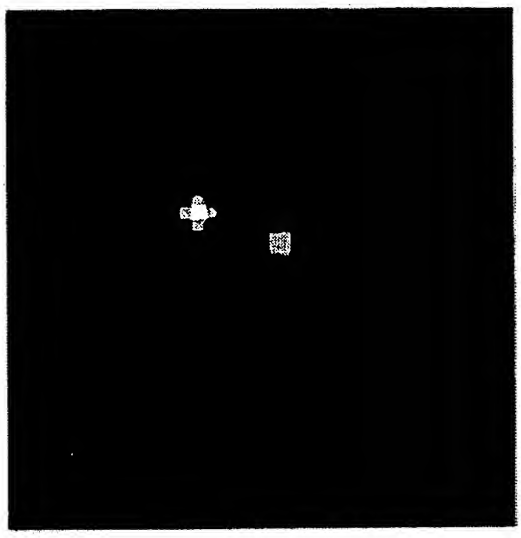


FIG-5b

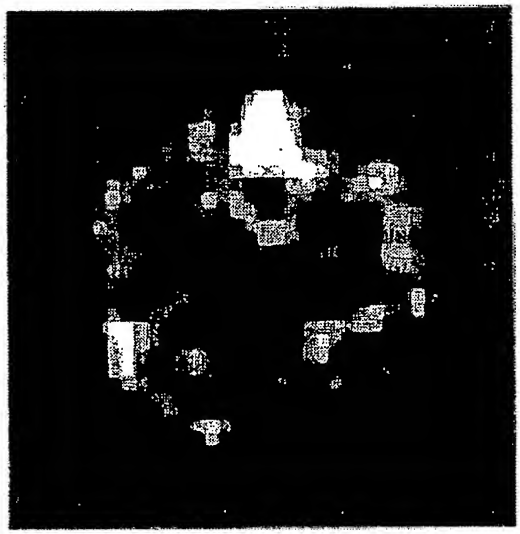


FIG-5c

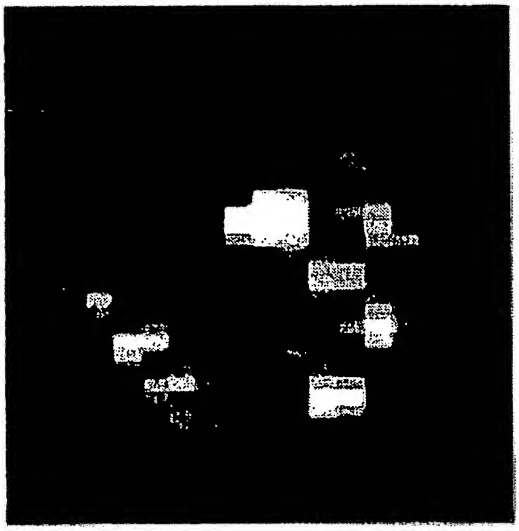


FIG-5d

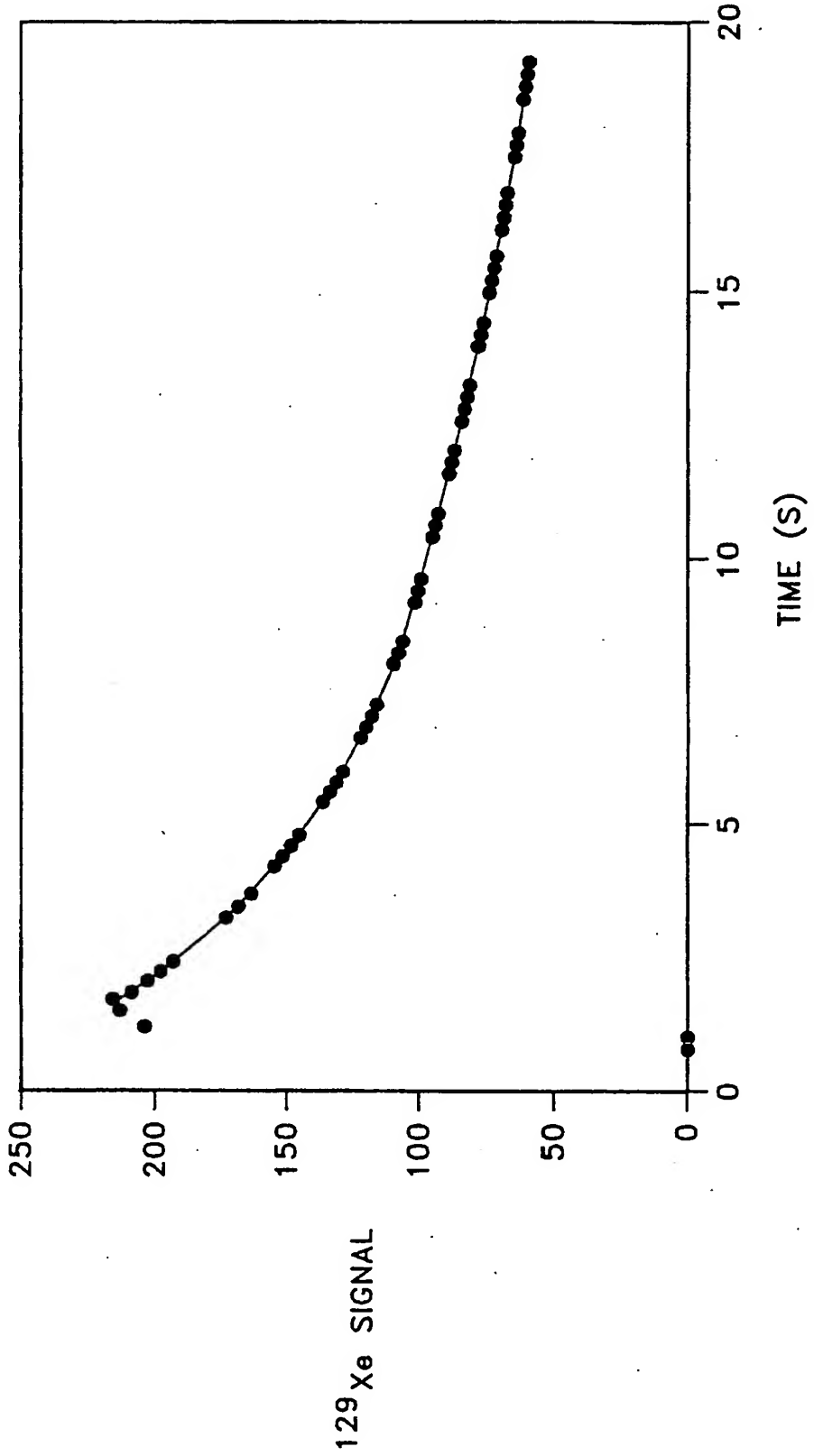
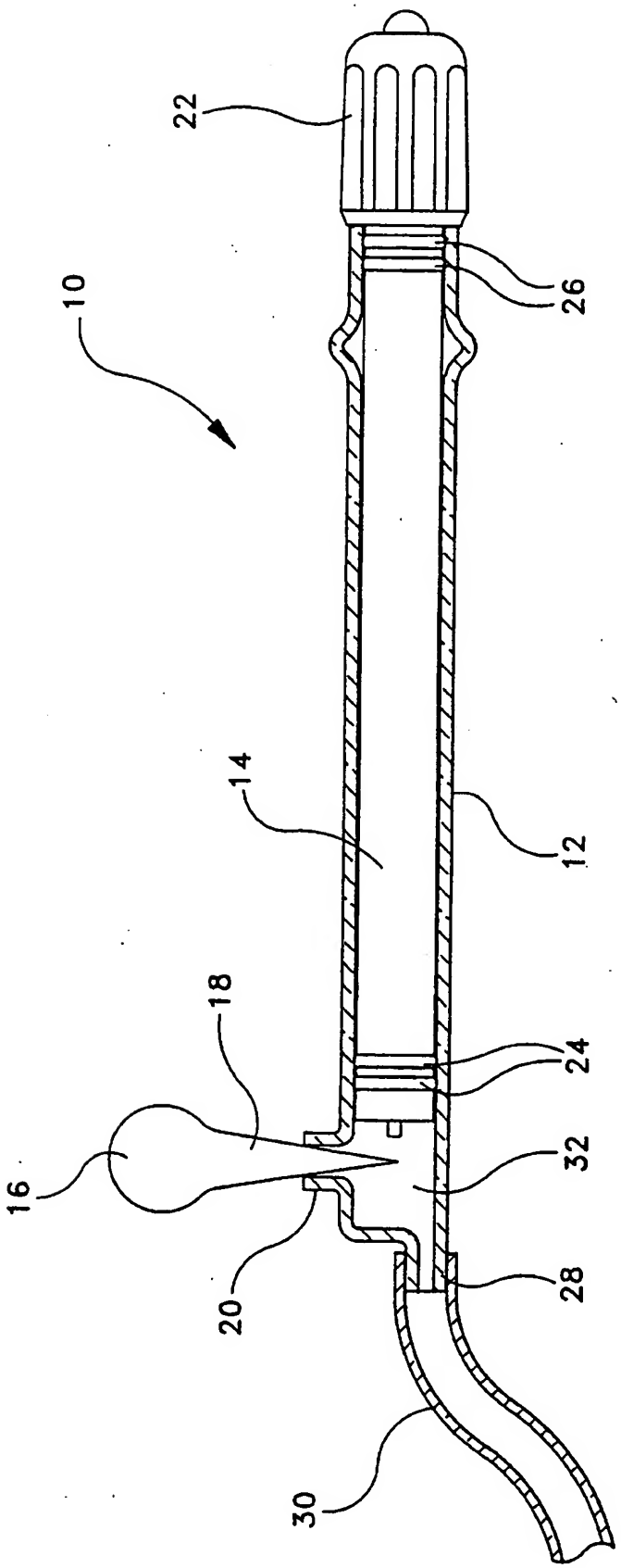


FIG-6



FIG—7

MAGNETIC RESONANCE IMAGING USING HYPERPOLARIZED NOBLE GASES

This invention was made with government support under grant numbers 88-0165 and F49620-92-J-0211, awarded by the Air Force Office of Scientific Research. The government has certain rights in this invention.

BACKGROUND OF THE INVENTION

The present invention relates generally to techniques of nuclear magnetic resonance imaging. In particular, the present invention relates to, among other things, the detection and imaging of a noble gas by nuclear magnetic resonance spectrometry.

Current views as to the molecular basis of anesthetic action are mostly derived from experimental work carried out in vitro. Interpretation of many of the results of these studies are extremely controversial, e.g., changes in lipid structure are observed at exceedingly high, indeed toxic, concentrations of anesthetic. Changes observed in vitro, from animals whose physiology has been altered, or from animals administered non-clinical doses of anesthetics might not reflect the effects of these agents clinically. It is believed that significant progress can be made by employing direct non-invasive methods for the detection and characterization of anesthetics in living animals. Both lipid solubility and protein binding undoubtedly do play a role, but new ideas are now needed.

Attempts have been made to bring powerful nuclear magnetic resonance (NMR) techniques to bear on this problem. (References 1–3). Wyrwicz and co-workers pioneered the use of fluorine-19 (^{19}F) NMR spectroscopy to observe fluorinated anesthetics in intact tissues and recorded the first ^{19}F NMR spectra from the brain of a live anesthetized rabbit. (References 1, 4). These early studies demonstrated the feasibility of studying the fate of anesthetics in live mammals. Burr and collaborators also used halothane and other fluorinated anesthetics for monitoring membrane alterations in tumors by ^{19}F NMR. (References 5–6). In recent years, several groups have conducted ^{19}F NMR studies which have shed light on the molecular environment of anesthetics in the brains of rabbits and rats. (References 3, 7). Using a surface coil placed on top of the calvarium during halothane inhalation, two overlapping spectral features observed by d'Avignon and coworkers, perhaps 0.1–0.2 ppm apart, could be resolved through their different transverse relaxation times (T_2). (Reference 3). The biexponential dependence of the spin-echo amplitude on echo delay reported in this study demonstrated that anesthetics in different molecular environments could be discerned in the brain in vivo using ^{19}F NMR. Such environments, separated by chemical shifts of only about 0.1 ppm, had previously been reported by Wyrwicz et al. in high resolution studies of excised neural tissue. (Reference 4).

Notwithstanding such attempts to use other compounds for NMR imaging, state-of-the-art biological magnetic resonance imaging (MRI) has remained largely restricted to the water proton, ^1H_2O, NMR signal. The natural abundance of water protons, about 80–100 M in tissue, and its large magnetic moment make it ideal for most imaging applications. Despite its tremendous value as a medical diagnostic tool, however, proton MRI does suffer several limitations. Most notably, the water protons in lung tissue, and the protons in lipids of all interesting biological membranes, are notoriously NMR invisible as a result of the short T_2 in such

environments. (References 8–9). Other ^1H signals and signals from other biologically interesting nuclides are either present in too low a concentration (10^{-3} to 10^{-1} M, compared to ca. 100 M for H_2O) or have undesirable NMR characteristics. In studying dynamic processes with ^1H_2O, one must sacrifice much of the proton signal to exploit differences in effective spin density resulting from T_1 and/or T_2 spatial variation. (Reference 10).

Various noble gases are known to be effective anesthetic agents. For example, Xenon is approved for use in humans, and its efficacy as a general anesthetic has been shown. Attempts have previously been made to take advantage of the properties of Xenon for purposes of medical imaging, but success has heretofore been extremely limited, and techniques have been impractical at best. For example, the ^{127}Xe isotope was used in early ventilation studies of the lung. (References 11–12). Unfortunately, the poor image quality attained limited its clinical use. Xenon has, however, been used as a contrast enhancement agent in computed tomography (CT) studies of the brain, (References 13–14), and as a tracer for regional cerebral blood flow (rCBF) measurements. (Reference 15).

An isotope of Xenon, Xenon-129 (^{129}Xe), has non-zero nuclear spin (i.e., ½) and therefore is a nucleus which, in principle, is suited to study by nuclear magnetic resonance techniques. Despite the apparent potential for use of Xenon in magnetic resonance imaging, its small magnetic moment, and the low number densities of gas generally achievable, have heretofore been insuperable obstacles to practicable magnetic resonance (MR) imaging of ^{129}Xe at normal, equilibrium (also known as "Boltzmann") polarizations, P ($P\sim10^{-5}$ in 0.5–1.5 Tesla (T) clinical imaging systems). However, unlike the water proton (^1H) employed as the nucleus in conventional NMR techniques, the nuclear magnetic resonance signals obtainable from ^{129}Xe are extraordinarily sensitive to local environment and therefore very specific to environment.

Certain aspects of the behavior of ^{129}Xe, and other noble gas isotopes having nuclear spin, in various environments have been studied and described. For example, Albert et al. have studied the chemical shift and transverse and longitudinal relaxation times of Boltzmann polarized ^{129}Xe in several chemical solutions. (Reference 16). Albert et al. and others have also shown that Oxygen can affect longitudinal relaxation time T_1 of ^{129}Xe. (References 17–18). Miller et al. have also studied the chemical shifts of ^{129}Xe and ^{131}Xe in solvents, proteins, and membranes. (Reference 2). However, none of these publications provides any indication of a method by which ^{129}Xe could be used for nuclear magnetic resonance imaging.

It is known in the art that the polarization of certain nuclei, such as noble gas nuclei having nuclear spin, may be enhanced over the equilibrium or Boltzmann polarization, i.e., hyperpolarized. Such techniques include spin exchange with an optically pumped alkali metal vapor and metastability exchange.

The physical principles underlying the hyperpolarization of noble gases have been studied. (Reference 19). For example, Happer et al. have studied the physics of spin exchange between noble gas atoms, such as Xenon, with alkali metals, such as Rubidium. (Reference 20). Others have studied spin exchange between Helium and alkali metals. (References 21–22, 49). Other publications have described physical aspects of spin exchange between alkali metals and noble gases. (References 23–24). The technique of using metastability exchange to hyperpolarize noble gases

has been studied by Schearer et al. and by Hadeishi et al. (References 26–31).

Other publications, by Cates et al. and Gatzke et al., describe certain behaviors of frozen, crystalline ^{129}Xe that has been hyperpolarized. (References 32–33). Cates et al. and others describe spin-exchange rates between Rubidium and ^{129}Xe at high Xenon pressures as measured by magnetic resonance apparatus. (References 34–35). These publications, however, relate to ^{129}Xe behavior in highly controlled physical systems and provide no description concerning how ^{129}Xe might be used to produce images by nuclear magnetic resonance.

Raftery et al. have described optically pumped ^{129}Xe as an adsorption probe for the study of surface structure by analysis of NMR spectra. (References 36–37). Long et al. have also observed the chemical shift of laser polarized Xenon adsorbed to a polymer surface. (Reference 38).

U.S. Pat. Nos. 4,856,511 and 4,775,522 to Clark describe a nuclear magnetic resonance technique for detecting certain dissolved gases in an animal subject. Gas compositions described as useful for this technique include fluorine compounds such as perfluorocarbons. Other gases suggested to be potentially useful for the technique of Clark include ^{129}Xe but Clark fails to recognize any of the difficulties which have heretofore rendered use of ^{129}Xe for magnetic resonance imaging of biological subjects impracticable.

Therefore, it would be a significant advance in the art to overcome the above-described difficulties and disadvantages associated with nuclear magnetic resonance imaging, in a manner which would permit the imaging of noble gases, especially the imaging of noble gases in biological systems, without requiring excessively long image acquisition times and without being limited to systems and environments previously imageable only by ^{1}H NMR.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a method of performing nuclear magnetic resonance imaging which includes detecting the spatial distribution of at least one noble gas by nuclear magnetic resonance (NMR), and generating a representation of the noble gas spatial distribution.

In a preferred embodiment, there is also provided a method of performing nuclear magnetic resonance imaging of an animal or human subject by administering an imageable amount of at least one noble gas to the subject, employing an NMR imaging spectrometer to detect radio-frequency signals derived from the magnetic resonance of at least one noble gas, processing the detected signals to obtain an NMR parameter data set as a function of the spatial distribution of at least one noble gas, and further processing the data set to generate a representation of at least one dimension of the spatial distribution of at least one noble gas.

In another preferred embodiment, the method of the invention further includes detecting and imaging at least one hyperpolarized noble gas. The hyperpolarized noble gas is preferably hyperpolarized by laser polarization through spin exchange with an alkali metal or by metastability exchange. The noble gas is preferably selected from among Helium-3, Neon-21, Krypton-83, Xenon-129, Xenon-131 and mixtures thereof. Most preferably, the noble gas is Helium-3 or Xenon-129. Combinations of noble gases and/or noble gas isotopes are contemplated, as are combinations of hyperpolarized and non-hyperpolarized noble gases and/or noble gas isotopes. When the noble gas is laser polarized through spin exchange with an alkali metal, preferably an alkali metal selected from among Sodium-23, Potassium-39, Cesium-133, Rubidium-85, and Rubidium-87. Most preferably, the alkali metal is Rubidium-85 or Rubidium-87.

The method of the invention preferably includes detecting and imaging at least one physical dimension of the spatial distribution of at least one noble gas, more preferably including detecting and imaging two or three physical dimensions. The method of the invention may also include detecting and imaging alterations of the spatial distribution of the noble gas as a function of time.

The generating of a representation of a noble gas preferably includes generating a representation of at least one physical dimension of the spatial distribution of the noble gas, more preferably including generating a representation of two or three physical dimensions of the noble gas. The generating of the representation may also include generating a representation of one or more physical dimensions of the spatial distribution of the noble gas as a function of time, including such NMR parameters as chemical shift, T_1 relaxation, T_2 relaxation, and $T_{1\rho}$ relaxation. Preferably, the method of the invention includes generating a visual representation.

The noble gas being imaged is preferably distributed spatially in at least one physical phase such as a gas, liquid, gel, or solid. The noble gas may be imaged as distributed in two or more physical phases in one sample. The noble gas being imaged may be distributed on a solid surface. The noble gas may be imaged in association with various materials or environments.

The sample being imaged using a noble gas may include an in vitro chemical, in vitro biological or in vivo biological, system. When the noble gas distribution in an in vivo biological system is imaged, the system may include one or more human or animal subjects. The noble gas is preferably distributed in an organ or body system of the human or animal subject. Alternatively, the noble gas may be distributed in an anatomical space of the subject.

In another embodiment of the invention, there is provided a medical composition which includes a medically acceptable bifunctional gas effective for in vivo anesthesiological and nuclear magnetic resonance imaging functions. In a preferred embodiment, the gas composition includes at least one noble gas, preferably selected from among Helium-3, Neon-21, Krypton-83, Xenon-129, and Xenon-131. More preferably, the noble gas is Helium-3 or Xenon-129. The noble gas is preferably hyperpolarized, more preferably through spin exchange with an alkali metal or through metastability exchange. Combinations of hyperpolarized and non-hyperpolarized noble gases and noble gas isotopes are possible.

Also in accordance with the present invention, there is provided apparatus for nuclear magnetic resonance imaging which includes NMR imaging means, for detecting and imaging at least one noble gas, and means for providing imageable quantities of the noble gas. In a preferred embodiment, the apparatus includes means for providing imageable quantities of a hyperpolarized noble gas. The apparatus of this embodiment includes hyperpolarizing means, preferably means for hyperpolarizing a noble gas through spin exchange with an alkali metal or through metastability exchange. The noble gas may be provided in continuous, discontinuous, and/or quasi-continuous mode, and when more than one noble gas is provided, noble gases may be provided as a mixture or individually, and may be provided

either together or by separate routes and/or at separate times and durations.

The noble gas may be contacted with the sample to be imaged in gaseous, liquid, or solid form, either alone or in combination with one or more other components in a gaseous, liquid, or solid composition. The noble gas may be combined with other noble gases and/or other inert or active components. The noble gas may be delivered as one or more boluses or by continuous or quasi-continuous delivery.

Also in accordance with the invention there is provided a method of performing nuclear magnetic resonance imaging of a human or animal subject. In this embodiment, the method includes administering to a subject an imageable amount of a hyperpolarized noble gas, generating radio-frequency signals from the nuclear magnetic resonance of the hyperpolarized noble gas by means of a nuclear magnetic resonance imaging spectrometer, detecting the generated radio-frequency signals, processing the detected radio-frequency signals to derive a nuclear magnetic resonance parameter data set as a function of a spatial distribution of the hyperpolarized noble gas in the subject, and further processing said nuclear magnetic resonance parameter data set to derive a representation corresponding to at least one spatial dimension of the spatial distribution of the hyperpolarized noble gas in the subject.

The noble gas may be administered to a human or animal subject as a gas or in a liquid, either alone or in combination with other noble gases and/or other inert or active components. The noble gas may be administered as a gas by either passive or active inhalation or by direct injection into an anatomical space such as lung or gastrointestinal tract. The noble gas may be administered as a liquid by enteral or parenteral injection. The preferred method of parenteral administration includes intravenous administration, optionally by contacting blood with the noble gas extracorporeally and reintroducing the noble gas-contacted blood by intravenous means.

The present invention solves the disadvantages inherent in the prior art by providing a method for imaging at least one noble gas by nuclear magnetic resonance. The present method provides a new and unexpectedly powerful method of NMR imaging of noble gas spatial and temporal distribution in non-biological as well as in in vitro and in vivo biological systems. The present invention also permits the acquisition of images of high signal to noise ratio, in unexpectedly short acquisition periods. In addition, the present invention provides a method for imaging biological phenomena of short duration as well as for imaging systems previously not amenable to imaging by conventional ^{1}H NMR techniques.

Other objects and advantages of the present invention will become more fully apparent from the following disclosure, figures, and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1*a* shows a nuclear magnetic resonance spectrum of ^{129}Xe in a rat brain synaptosome suspension; FIG. 1*b* shows a nuclear magnetic resonance spectrum of ^{129}Xe in a homogenate of rat brain tissue; FIG. 1*c* shows a nuclear magnetic resonance spectrum of ^{129}Xe in a whole rat brain preparation.

FIG. 2*a* shows a graphical representation of a glass sphere 20 mm in diameter; and FIG. 2*b* shows a nuclear magnetic resonance image of Boltzmann polarized ^{129}Xe gas in a 20 mm diameter glass sphere.

FIG. 3*a* shows a graphical representation of a glass sphere, 20 mm in diameter, containing octanol (shaded region) and Xenon gas (unshaded region); FIG. 3*b* shows a nuclear magnetic resonance spectrum illustrating NMR signals obtained from ^{129}Xe in gas phase and in octanol; FIG. 3*c* shows a nuclear magnetic resonance image of ^{129}Xe in octanol in 20 mm glass sphere; and FIG. 3*d* shows a nuclear magnetic resonance image of ^{129}Xe in gas phase in a 20 mm glass sphere.

FIG. 4 shows a series of nuclear magnetic resonance images of a hyperpolarized ^{129}Xe gas phantom, representing different mutually parallel planes.

FIGS. 5*a*–5*c* show a sequence of nuclear magnetic resonance images of a mouse lung inflated with hyperpolarized ^{129}Xe gas; and FIG. 5*d* shows a nuclear magnetic resonance image of ^{1}H in a mouse heart.

FIG. 6 shows a graph illustrating a decrease in ^{129}Xe magnetic resonance signal intensity, obtained from mouse lung inflated by hyperpolarized ^{129}Xe, as a function of time.

FIG. 7 shows a longitudinal section view of a noble gas delivery device for nuclear magnetic resonance imaging of noble gases.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Nuclear magnetic resonance spectroscopy is a technique which is well known in a wide variety of scientific disciplines. Basic considerations regarding the conventional practice of nuclear magnetic resonance imaging, especially as applied to biological systems, are found in Rinck et al., *An Introduction to Magnetic Resonance in Medicine* (1990), especially Chapters 1–4 (Reference 39); and Wehrli, F. W., "Principles of Magnetic Resonance", Chapter 1, and Wood, M. L., "Fourier Imaging" Chapter 2, in *Magnetic Resonance Imaging*, Vol. 1, 2d ed., Stark et al., eds. (1992) (Reference 40). These publications are incorporated by reference herein. In certain disciplines, an adaptation of NMR spectroscopy, involving the generation of images from NMR data has found increasing popularity. In medicine, certain MRI techniques have become fairly commonplace, principally employing the water proton ($^{1}H_2O$) for the imaging of certain regions in the body.

Nonetheless, certain other magnetically susceptible nuclei are desired to be adapted for MRI for various reasons. In particular, the physical characteristics of other elements may predispose the nuclei to the imaging of other kinds of physical and biological systems. In medicine, other nuclei are desired which can enable the imaging of regions of the body which are difficultly accessible by currently available NMR probes. Prior to the unexpected observations of the utility of noble gases for MRI applications, as described herein, acceptable alternative nuclear probes have been unavailable.

Noble gas isotopes having non-zero nuclear spin have now been discovered to offer vast possibilities for use in MRI. For example, the ^{129}Xe isotope is, in principle, suited to NMR uses, but is 26% naturally abundant and has a sensitivity relative to ^{1}H (in conventional NMR) of 2.12×10^{-2}. The resonance frequency of ^{129}Xe spans an enormous range (0–300 ppm) over the gas and condensed phase, and is exceptionally sensitive to chemical environment. (Reference 2). Its longitudinal relaxation time, T_1, is huge (practically at least 3000 s in the pure gas phase, and theoretically perhaps as long as 56 hrs at 1 atm), (References 32, 41), and is particularly sensitive to chemical environment, O_2 con-

centration, (References 17–18), and the effects of other relaxation promoters. (References 2, 42, 16). Its transverse relaxation time is also susceptible to relaxation promoters. (References 16, 18, 43).

The longitudinal and transverse relaxation times, T_1 and T_2, respectively, are also indicative of the environment surrounding the ^{129}Xe atom, e.g., whether the atom is bound to a protein, dissolved in a lipid, or constrained in some other way. Thus a combination of chemical shift, T_1, and T_2 data can provide a basis for distinguishing the presence or absence of the nucleus in a particular environment as well as for identifying the nature of the environment in question.

Elemental Xenon is a benign and effective anesthetic, (Reference 44), which is not metabolized by the body. Xenon has an essentially Raoult's Law solubility in non-polar solvents. (Reference 45). Inhaled into the lungs, Xenon equilibrates quickly with the pulmonary circulation, reaching a steady state with the entire blood volume in one blood circuit, (Reference 13), on average, about 1 s or 1–2 breaths in the mouse, about 18 s in humans. (Reference 46). Xenon is known to accumulate rapidly in highly-vascularized tissue. For example, in the brain, which contains 10% lipid and 10% protein, (Reference 10), one can expect steady-state concentrations (for 0.5–1.0 atm lung Xenon) of 5–10 mM in the membrane bilayers, 2–4 mM in water, and about 1–5 mM bound to proteins. (References 5, 47–48). Xenon is also approximately twice as soluble in white matter as in gray matter. (Reference 13). The NMR resonance frequency of ^{129}Xe is different in each of the above sites, and exchange between compartments is slow on the chemical shift NMR timescale. (References 2, 16–17). The potential usefulness of hyperpolarized ^{129}Xe as a contrast agent in biological systems is therefore apparent.

The total Xenon concentration in materials of biological interest will typically range between about two and about five times its solubility in pure water. The problem with any attempt to image Boltzmann polarized Xenon in such a system is that many samples are required in order to determine a solution parameter. These difficulties stem in large part from the lower concentrations of ^{129}Xe, its smaller magnetic moment, and its lower natural abundance as compared with ^1H_2O. Similar considerations apply with regard to other noble gases which are generally less soluble in water as well as in non-polar media.

For example, the spectrum of FIG. 1*c*, obtained in 8 hrs from in vitro brain samples taken from rats anesthetized with Xenon gas, has significantly less signal to noise (S/N) than a spectrum of ^{129}Xe in a synaptosomal suspension shown in FIG. 1*a*, obtained in 27 hrs under a Xe pressure of 3 atm.

The difficulties which have heretofore prevented the development of noble gas MRI are clear: typically long longitudinal relaxation times and low signal strength require signal averaging of exceedingly many free induction decays (FIDs) over long periods of time. It is clear that to conduct in vivo NMR experiments, extraordinary enhancement of the noble gas signal is necessary. The total accumulation times for Boltzmann noble gas spectra is prohibitively long in such biological samples.

The ability to use noble gases for NMR imaging, then, is directly and profoundly limited by the average signal intensity and the signal acquisition ability of the spectrometer. Given current NMR spectrometer technology, it is reasonable to conclude that on the order of a 10,000 fold increase in sensitivity, e.g., that increase necessary to render Xenon imaging possible using Boltzmann polarized Xenon, could take years if not decades to develop, assuming it is feasible at all. The required sensitivity increase is more practicably attained through hyperpolarization, for example, through the use of optical pumping and spin exchange, (References 21–22, 32, 36), or metastability exchange. (References 26–31). This method of enhancing the noble gas signal can be used to create noble gas nuclear polarizations which are on the order of 10^4–10^6 larger than typical thermal equilibrium polarizations. Nuclear polarizations attained using these techniques are easily of order 0.25, (Reference 32), and can approach 1.0, making the product of spin density and polarization at least an order of magnitude larger than for the proton (^1H) in typical imaging situations. Thus, it has now been unexpectedly found that the hyperpolarization of noble gases permits a spectacular new means of producing magnetic resonance images.

While the extraordinary property of hyperpolarizability of noble gases, especially ^{129}Xe and ^3He, is of great importance in rendering the imaging of biological systems possible, other factors play a role in developing such images. For example, noble gases exhibit other unusual properties, including distinctly different behavior compared to ^1H_2O in (a) cell and tissue compartmentalization; (b) dramatically time-dependent distribution; and (c) response of resonance frequency, T_1, and T_2 to environment, O_2 concentration and subcellular exchange kinetics. The combination of hyperpolarizability of noble gases and these other unusual properties enables the use of noble gases as a new and qualitatively different source of NMR image contrast. For example, as opposed to water protons, ^{129}Xe is not omnipresent; its space and time distribution in the body depends entirely on the anatomy and physiology of Xenon transport. (Reference 13). This permits its use in magnetic resonance imaging (MRI) and magnetic resonance spectrometry (MRS) studies of soft-tissue anatomy, physiology (e.g., cerebral blood flow, cerebral activity) and pathology (e.g., demyelination, early detection of tumors or other foci of changed or anomalous metabolism). Moreover, the large MR signal strengths obtainable using hyperpolarized noble gases permit the use of the high-speed imaging protocols, which have heretofore been possible only with ^1H_2O.

The imaging method of the invention is preferably performed using the ^{129}Xe and/or the ^3He nuclei. However, the method of the invention may also be performed with other noble gases, i.e., other noble gas isotopes having nuclear spin. ^3He, ^{129}Xe and the other noble gases may be preferred in different applications because of their different physical and magnetic resonance properties. A list of noble gas nuclei useful according to the invention is provided below in Table I. This list is intended to be illustrative and non-limiting.

TABLE I

Hyperpolarizable Noble Gases		
Isotope	Natural Abundance (%)	Nuclear Spin
^3He	$\sim 10^{-6}$	1/2
^{21}Ne	0.27	3/2
^{83}Kr	11.5	9/2
^{129}Xe	26.4	1/2
^{131}Xe	21.2	3/2

While each of the noble gas isotopes listed in Table I, alone or in combination, may be used for nuclear magnetic resonance imaging according to the invention, it is known that the degree of polarization of the gases in equilibrium (Boltzmann) state is prohibitively low, preventing high speed image acquisition. The various parameters governing

signal decay such as T_1 and T_2 relaxation and the local environment of the nucleus will also determine whether high speed images can be effectively acquired. These limitations become of great importance in acquisition of images from in vitro and in vivo biological systems since the time course of events desired to be imaged often requires data acquisition periods of less than one second. Enhancement of the NMR signal is, therefore, highly desirable. Accordingly, the noble gas is preferably hyperpolarized relative to its normal Boltzmann polarization. Such hyperpolarization is preferably induced prior to data acquisition by an NMR spectrometer and may be induced by any of the techniques known in the art.

Further enhancement of the noble gas magnetic resonance signal may be obtained, independently of, or together with, hyperpolarization, by increasing the proportion of the imageable isotope in each noble gas to a level above the natural abundance of such imageable isotopes in the noble gas. In the case of ^{129}Xe which has a natural isotopic abundance of about 26%, this amounts to enhancement by no more than a factor of four, even in a gas which is enriched to 100% ^{129}Xe. Other considerations, such as the hyperpolarizability of the noble gas, usually play a much larger role in signal enhancement, but isotopic enrichment can provide a significant contribution to the ultimate efficacy of the present invention. This is especially true in the case of ^3He which has a natural abundance of on the order of 10^{-6}. Even the hyperpolarizability of ^3He and its very large magnetic resonance signal could be considerably offset by the low natural abundance of this isotope. Despite its low natural abundance, however, ^3He is readily available in very pure form as a result of industrial use of tritium (^3H) which decays exclusively to ^3He. The ready availability of artificial sources of ^3He eliminates concerns regarding its low natural abundance and associated expensive enrichment processes.

Noble gases may be hyperpolarized for use according to the invention through any of various means known in the art, such as spin-exchange interactions with optically pumped alkali metal vapor. (References 34–35, 49–50). The optical pumping and spin-exchange can be performed in the absence of an applied magnetic field, but is preferably performed using modest fields of about 1 G or larger. Pumping in the NMR magnet bore at fields of several Tesla is also possible. The maximum steady state ^{129}Xe nuclear polarization achievable depends on the time constant characterizing the spin exchange with the alkali metal and the time constant characterizing the relaxation (T_1) due, for example, to contact with the surfaces of the pumping cell. For instance, with $T_1 \approx 20$ min, polarizations of 20–40% are quite practicable, (Reference 32), and polarizations of 90% or more should be attainable. The long T_1 of the gas also allows samples to be manipulated, even stored as Xe ice, (Reference 32), and transported on time scales of hours or even days, without serious loss of magnetization.

The art of hyperpolarizing noble gases through spin exchange with an optically pumped alkali-metal vapor starts with the irradiation of the alkali-metal vapor with circularly polarized light at the wavelength of the first principal (D_1) resonance of the alkali metal (e.g. 795 nm for Rb) The $^2S_{1/2}$ ground state atoms are thus excited to the $^2P_{1/2}$ state and subsequently decay back to the ground state. If performed in a modest (10 Gauss) magnetic field aligned along the axis of incident D_1 light, this cycling of atoms between the ground and first excited states leads to nearly 100% polarization of the atoms in a few microseconds. This polarization is carried mostly by the lone valence electron characteristic of all alkali metals; this essentially means that all of these electrons have their spin either aligned or anti-aligned to the magnetic field depending upon the helicity (right- or left-handed circular polarization state) of the pumping light. If a noble gas with non-zero nuclear spin is also present, the alkali-metal atoms can undergo collisions with the noble gas atoms in which the polarization of the valence electrons is transferred to the noble-gas nuclei through a mutual spin flip. This spin exchange results from the Fermi-contact hyperfine interaction between the electron and the noble-gas nucleus. By maintaining the alkali-metal polarization at nearly 100% with the pumping light, large non-equilibrium polarizations (5%–80%) are currently achievable in large quantities of a variety of noble gases through this spin-exchange process. For example, one currently available Titanium:Sapphire-laser could theoretically provide 1 g/hr (200 cc-atm/hr) of highly polarized ^{129}Xe.

The alkali metals capable of acting as spin exchange partners in optically pumped systems include any of the alkali metals. Preferred alkali metals for this hyperpolarization technique include Sodium-23, Potassium-39, Rubidium-85, Rubidium-87, and Cesium-133. Alkali metal isotopes, useful according to the invention, and their relative abundance and nuclear spins are listed in Table II, below. This list is intended to be illustrative and non-limiting.

TABLE II

Alkali Metals Capable of Spin Exchange

Isotope	Natural Abundance (%)	Nuclear Spin
^{23}Na	100	3/2
^{39}K	93.3	3/2
^{85}Rb	72.2	5/2
^{87}Rb	27.8	3/2
^{133}Cs	100	7/2

Alternatively, the noble gas may be hyperpolarized using metastability exchange. (References 28, 51). The technique of metastability exchange involves direct optical pumping of, for example, ^3He without need for an alkali metal intermediary. The method of metastability exchange usually involves the excitation of ground state ^3He atoms (1^1S_0) to a metastable state (2^3S_1) by weak radio frequency discharge. The 2^3S_1 atoms are then optically pumped using circularly polarized light having a wavelength of 1.08 μm in the case of ^3He. The light drives transitions up to the 2^3P states, producing high polarizations in the metastable state to which the 2^3P atoms then decay The polarization of the 2^3S_1 states is rapidly transferred to the ground state through metastability exchange collisions between metastable and ground state atoms. Metastability exchange optical pumping will work in the same low magnetic fields in which spin exchange pumping works. Similar polarizations are achievable, but generally at lower pressures, e.g., about 0–10 Torr.

The method of the invention preferably includes detecting and imaging at least one physical dimension of the spatial distribution of at least one noble gas, more preferably including detecting and imaging two or three physical dimensions. The method of the invention may also include detecting and imaging alterations in the spatial distribution of the noble gas as a function of time.

The generating of a representation of a noble gas preferably includes generating a representation of at least one physical dimension of the spatial distribution of the noble gas, more preferably including generating a representation of two or three physical dimensions of the noble gas. The generating of the representation may also include generating

a representation of one or more physical dimensions of the spatial distribution of the noble gas as a function of time, including such NMR parameters as chemical shift, T_1 relaxation, T_2 relaxation and $T_{1\rho}$ relaxation. Preferably, the method of the invention includes generating a visual representation.

Representations of the spatial distribution of a noble gas may be generated by any of the methods known in the art, subject to the type of information desired to be represented. These techniques employ various means for collecting and manipulating nuclear magnetic resonance data for the generation of images. Such methods are described in the literature available in the art and include, without limitation, Fourier imaging, planar imaging, echo planar imaging, projection-reconstruction imaging, spin-warp Fourier imaging, gradient recalled acquisition in the steady state (GRASS) imaging also known as fast low angle shot (FLASH) imaging, and hybrid imaging.

Such imaging methods are described in, for example, Ernst et al., *Principles of Nuclear Magnetic Resonance in One and Two Dimensions* (1987) (Reference 52), particularly Chapter 10, "Nuclear Magnetic Resonance Imaging", pages 539–564; Shaw, D. D., "The Fundamental Principles of Nuclear Magnetic Resonance", Chapter 1 in *Biomedical Magnetic Resonance Imaging*, S. W. Wehrli et al. eds. (1988) (Reference 53); and Stark et al. eds., *Magnetic Resonance Imaging*, Vol. 1, 2d ed. (1992) (Reference 40). These publications are incorporated herein by reference.

The selection of imaging method will depend on the behavior of the noble gas nucleus under investigation, the nature of the sample and the degree of interaction of the nucleus with the sample. The selection of imaging method will also depend on whether one or more spatial dimensions of the spatial distribution of the noble gas is desired to be represented and whether a temporal or time-dependent dimension is desired to be represented. When a multidimensional representation is desired such representation may be generated by, for example, multi-slice imaging or volume imaging.

It is generally preferred that the image or representation be generated by a method which is as fast and as sensitive as possible. Preferred imaging methods include the FLASH or GRASS imaging method and the echo-planar imaging (EPI) method. These methods are preferred for their capacity to generate images through fast data acquisition, thereby conserving polarization of the noble gas. EPI is especially preferred because it is a relatively fast method and requires only one radio-frequency (RF) pulse per image. It thus permits maximum utilization of the available polarization. These preferred methods also permit fast time resolution of time-dependent phenomena in human and animal subjects. Such applications include, for example, magnetic resonance angiography (MRA) studies, functional imaging of the nervous system (e.g., brain), as well as studies of variations in cardiopulmonary and circulatory physiological states.

The nuclear magnetic resonance imaging method of the invention also includes the registration of multiple imaging modalities. For example, using coils tunable to ^{129}Xe frequencies and the frequencies of one or more other magnetic probes permits enhanced data interpretation. Such combined multiple imaging approaches would include, for example the combined imaging of ^{129}Xe with ^{1}H, and the imaging of more than one noble gas, such as imaging of ^{129}Xe with ^{3}He. In this embodiment, geometric image registry and overlay are possible, including the generation of false-color images, in which distinct colors would represent distinct probes. Image subtraction techniques would also be possible using combinations of ^{129}Xe with other probes, or combinations of noble gas probes.

The noble gas being imaged is preferably distributed spatially in at least one physical phase such as a gas, liquid, gel, or solid. The noble gas may be imaged as distributed in two or more physical phases in one sample. The noble gas being imaged may be distributed on a solid surface. The noble gas may be imaged in association with various materials or environments such as, without limitation, zeolites, xenon clathrates, xenon hydrates, and polymers.

The sample being imaged using a noble gas may include an in vitro chemical, in vitro biological or in vivo biological, system. When the noble gas distribution in an in vivo biological system is imaged, the system may include one or more human or animal subjects. The noble gas is preferably distributed in an organ or body system of the human or animal subject, including, without limitation, lung tissue, nervous tissue, brain tissue, gastrointestinal tissue or cardiovascular tissue or combinations thereof. Alternatively, the noble gas may be distributed in an anatomical space such as, without limitation, lung space, gastrointestinal tract space, peritoneal space, bladder space or combinations thereof.

The noble gas may be contacted with the sample to be imaged in gaseous or liquid form, either alone or in combination with other components in a gaseous or liquid composition. The noble gas may be combined with other noble gases and/or other inert or active components. The noble gas may be delivered as one or more boluses or by continuous or quasi-continuous delivery.

In a preferred embodiment, there is also provided a method of performing nuclear magnetic resonance imaging of an animal or human subject by administering an imageable amount of a hyperpolarized noble gas to the subject, employing an NMR spectrometer to generate and detect radio-frequency signals derived from the magnetic resonance of the noble gas, processing the detected signals to obtain an NMR parameter data set as a function of the spatial distribution of the noble gas, and further processing the data set to generate a representation corresponding to at least one dimension of the spatial distribution of the noble gas.

The noble gas may be administered to a human or animal subject as a gas or as a liquid, either alone or in combination with other noble gases and/or other inert or active components. The noble gas may be administered as a gas by either passive or active inhalation or by direct injection into an anatomical space such as lung or gastrointestinal tract. The noble gas may be administered as a liquid by enteral or parenteral injection. The preferred method of parenteral administration includes intravenous administration, optionally by contacting blood with the noble gas extracorporeally and reintroducing the noble gas-contacted blood by intravenous means.

The cost of a purified noble gas tends to be relatively high as compared to the cost of common gases such as nitrogen or carbon dioxide. The cost is especially high in the case of Xenon which has been enriched to, for example, 70% ^{129}Xe. However, being inert, the noble gas is not metabolized in biological systems and can be recovered. For example, Xenon can be recovered from the exhaled breath of human subjects over about a 20 minute period. Such apparatus for noble gas recovery and repurification would include, for example, a cold trap and/or a zirconium getter apparatus, such as are known in the art. Other apparatus for recovery of noble gases may be employed.

It is preferred that, because of the high cost of the noble gas, the gas be maintained in a system which is substantially sealed to prevent loss to the atmosphere. Sealed containment apparatus would include a noble gas source, such as a gas canister or compressed gas tank, conduits to and away from a sample, as well as recovery apparatus.

The noble gas source may include a permanent or semi-permanent canister or pressurized containment apparatus. Alternatively, the noble gas may be supplied in disposable or refillable one-use containers such as pressurized gas ampoules or cylinders. The noble gas source may be integrated with a sealed noble gas supply and recovery system or may be stored separately and affixed to and opened to the supply and recovery system on a periodic or as-needed basis.

The sample to be studied, whether a physical structure, a chemical system, an in vitro system, a living animal or human host, or other suitable sample, is preferably imaged using apparatus which substantially prevents loss of Xenon to the environment, although the invention may be practiced without such apparatus. Thus, a sample may be imaged while maintained in a sample chamber substantially suffused or suffusable with the noble gas. Alternatively, for human or animal subjects, the subject may be fitted with an administration device, such as a sealed mask, for administration of the noble gas. In such cases, the sample chamber or noble gas administration device preferably communicates with a noble gas source and/or a noble gas recovery apparatus.

A hyperpolarized noble gas may be stored for extended periods of time in a hyperpolarized state. Storage systems capable of cryogenic storage of a hyperpolarized noble gas are preferably able to maintain temperatures such that noble gas is stored in frozen state. Frozen ^{129}Xe can be reasonably maintained at fields of $\geqq$500 Gauss at temperatures ranging from 4.2 K. (liquid helium temperature), for which T_1 is about a million seconds (10 days), to 77 K. (liquid nitrogen temperature), for which T_1 is about 10 thousand seconds. The fields necessary here may be provided by a small permanent magnet or by a larger electromagnet typically carrying on the order of ten or more amperes of current. For ^3He, things are quite different. Relaxation rates are such that low 10–20 Gauss fields can be used to hold it at room temperature-a few atmospheres will live for days under these conditions. The field here could also be a permanent magnet or a Helmholtz pair of coils carrying about one ampere of current. The conditions required for maintaining other hyperpolarized noble gases may be determined by those skilled in the art.

A noble gas which has been hyperpolarized by spin exchange with an alkali metal may be stored either before or after removal of any alkali metal used in spin exchange hyperpolarization techniques. In all cases in which rubidium or other alkali metal would interfere with the behavior of the system the alkali metal is removed before introduction of the noble gas to the sample. This removal of toxic alkali metal is important in biological samples and is especially critical in cases in which the sample is a living human or animal subject.

An alkali metal removal device may be employed either distant from the imaging site or proximally thereto. For example, the alkali metal removal device may be incorporated in a sealed noble gas administration system at a point prior to a conduit to a sample chamber or other administration device.

An alkali metal removal device would generally include a conduit for conducting the noble gas to a region or chamber which is cooler than the pumping region. At room temperature, the saturated vapor pressure of Rubidium, i.e., the pressure in an enclosure in the presence of a pool of liquid Rubidium, is about 10^{-9} atm. By moving the noble gas away from any macroscopic pools of liquid Rubidium, any remaining vapor is likely to plate out onto a cool (e.g., room temperature) surface, thereby never reaching an experimental subject. It is preferred, however, that a cold trap, such as is known in the art, be used.

The delivery of the noble gas to a sample may be performed as single or multiple bolus delivery. Such delivery would ordinarily be suited to the study of systems in which observations of the change in noble gas distribution is important. Such systems would include, inter alia, human or animal subjects in which an anatomical or physiological event or events are being examined as a function of time. Alternatively, the delivery of the noble gas to a sample may be performed as a continuous or quasi-continuous delivery. Such delivery would ordinarily be desired when steady state analyses of samples are desired. For example, high resolution imaging of human or animal organ systems would be possible by sequential imaging of steady state Xenon concentrations by data processing, e.g., image subtraction or signal averaging. Hyperpolarized Xenon or other noble gas could also be used as a marker or for contrast enhancement in whole body ^1H_2O NMR imaging in which the noble gas NMR signal could be digitally subtracted from the ^1H_2O NMR image For example, hyperpolarized Xenon could be introduced in the gastrointestinal tract of a subject to inflate the regions therein and to provide contrast enhancement when digital subtraction of signals is performed.

Comparative data have been obtained which illustrate the NMR behavior of ^{129}Xe in various environments. For example, various groups have determined chemical shift and relaxation rates (T_1 and T_2) for ^{129}Xe in environments such as n-octanol, benzene, water and myoglobin. (See References 2, 16). Octanol represents a relatively non-polar lipid-like environment resembling the interior of the cell membrane, water models aqueous regions, and the myoglobin solution represents a protein to which Xenon is known to bind. (Reference 54). The measured range of resonance frequencies for Xenon extends approximately 300 ppm over the gas and condensed phase. (Reference 2). Although the range of chemical shifts observed in these model biological systems is not as large as that in other solvents, it is large compared to the relevant ^{19}F brain resonance values that have been reported. (Reference 3).

Moreover, the huge range of T_1 values is extraordinary. Table III lists some reported values of T_1 and T_2 for ^{129}Xe in octanol, water and aqueous Fe(III) metmyoglobin (Reference 54); models representing two major cell compartments, lipid membrane and cytosol. The values for T_1 in octanol, 80 s, and water, 130 s, provide an indication of the extraordinarily long lifetimes of ^{129}Xe polarization (anoxic tissue with no other relaxers). In other biological environments, longer T_1 values are possible. The lower limit is unknown: The 5 ms T_1 in 10% Fe(III) metMb (a strong relaxer) implies a physiological lower limit much higher than this. The extremely short T_1 and T_2 values found for the protein solution certainly occur because Xenon binds very near the paramagnetic center of metmyoglobin. (Reference 54).

TABLE III

ENVIRONMENT	T_1(s)	T_2(s)	Δ^{129}Xe (ppm)*
Octanol	78.5	5.3	204.6
Water	131.3	5.3	195.3
Myoglobin	5.2×10^{-3}	0.57×10^{-3}	199.4
Benzene	160.5	0.88	196.4
Pure Gas Phase (1 atm)	56 hrs	≦56 hrs	0.4

*Shift relative to shift observed in pure gas at 0 atm.

The value of T_1 in benzene at 300° K., i.e., T_1=160 s, agrees well with that of Diehl and Jokisaari, i.e., T_1=155.0±6.2 s, at 9.4 T and 300° K., (Reference 43), rather than with the value of T_1=240 s obtained by Moschos and Reisse. (Reference 55). Measurements of T_1 and T_2 for ^{129}Xe are difficult to obtain, hence scarce. The values quoted here represent a significant fraction of the known list. The difficulties are obvious: typically, longitudinal relaxation times are long; low signal strength requires signal averaging of many free induction decay (FID) traces, hence very long overall accumulation times. The problem is particularly acute in aqueous systems: as noted above, the solubility of Xenon at 30° C., 0.5 atm, is 48 mM in octanol, but only 2.4 mM in water.

It would be desirable to investigate the possibility of observing multiple ^{129}Xe resonances within brain tissue, but the small signal from the small, largely aqueous brain volume of a live mouse, breathing an atmosphere of 50–70% normal Boltzmann-polarization ^{129}Xe, would require an enormous time interval of data collection for adequate signal averaging.

Seeking a system that would be tolerably stable for the necessary time interval, capable of being sealed with Xenon at 2–3 atm, but close enough to functioning brain cells, the behavior of Xenon in a synaptosome suspension has been studied. (Reference 16). Synaptosomes are presynaptic nerve terminals sheared away from their attachments to form resealed subcellular pseudocells that retain the morphology and chemical composition of the terminal nerve cell region, and much of the membrane functionality. Synaptosomes are rich in postsynaptic adhesions and constitute a source for postsynaptic membranes, synaptosomal mitochondria, transmitter receptors, and cleft material.

FIG. 1*a* shows a smooth, high S/N spectrum of 3 atm Xenon in equilibrium over a 10% (wet weight) rat brain synaptosome suspension as described by Albert et al. (Reference 16). This spectrum is resolution-enhanced with Gaussian broadening of 0.01 Hz and line broadening of −5.0 Hz. Two peaks can be seen; a broad resonance of about 3.4 ppm to higher frequency of a narrow component. The narrow peak appeared 0.33 ppm to higher frequency of that of ^{129}Xe in pure water, and is likely due to bulk magnetic susceptibility shift effects. Although collected using a simple one-pulse sequence, the spectrum required 27 hours of signal averaging to obtain the degree of signal strength and resolution shown.

An alternative model for investigating ^{129}Xe behavior in brain tissue has also been tested. FIG. 1*b* shows a ^{129}Xe spectrum obtained from a sample of rat brain homogenate as described by Albert et al. (See Reference 16). This spectrum also shows two resolved peaks; indicating that slow-exchange compartmentalization of ^{129}Xe in complex biological systems can also be observed. The decrease in high-field signal (aqueous ^{129}Xe) as compared to the synaptosomal spectrum (FIG. 1*a*) reflects a decrease in water content in the preparation. The spectrum of FIG. 1*b* required 8 hours of data accumulation, reflecting the difficulties inherent in attempting to examine ^{129}Xe in biological systems.

The behavior of ^{129}Xe in brain tissue has been studied by investigating whether any signal could be obtained from ^{129}Xe in whole rat brains. (See Reference 16). FIG. 1*c* shows a spectrum of ^{129}Xe obtained from a whole rat brain preparation, again showing two resolved peaks, but obtained with further decreased S/N. The two resolved peaks provide further evidence that ^{129}Xe is slow-exchange compartmentalized in complex biological systems. A further decrease in the proportion of high-field signal (aqueous ^{129}Xe) as compared to FIGS. 1*a* and 1*b*, reflects a further decrease in water content in this sample preparation. The spectrum required 8 hours of data accumulation, again illustrating the difficulty of obtaining NMR data from ^{129}Xe in biological systems.

It is known that ^{129}Xe, which has a long longitudinal relaxation time in the gas phase, can be relaxed by magnetic dipole-dipole interaction and/or Fermi-Contact interaction with the unpaired electron spins of dioxygen. (Reference 18). The solubility of Xenon (and also of dioxygen) in water is low. Due to the low sensitivity of the ^{129}Xe signal, the time required for determining the relaxivity of O_2 toward ^{129}Xe with a series of T_1 determinations over a range of O_2 concentrations in water would be prohibitively long.

The relaxivity of O_2 toward ^{129}Xe has been measured in only one liquid, i.e., octanol, which models an amphipathic membrane lipid. (Reference 17). The observed relaxivity, 0.029 $s^{-1}mM^{-1}$, is about three times larger than that estimated from previous reports for gas-phase relaxation, i.e., (Reference 18), 0.0087 $s^{-1}mM^{-1}$, as might be expected for encounters in the condensed phase. The dioxygen relaxivity for ^{129}Xe is constant over the concentration range studied, and thus $1/T_1$ will be a linear function of O_2 concentration over the entire physiological range (0–0.2 atm, 0–0.2 mM). This translates into a T_1 value of 18 s in air-saturated lipid, and 80 s in anaerobic lipid, in the absence of other relaxers. This is the first reported value for the O_2 relaxivity toward ^{129}Xe in a condensed phase. T_2 values over these O_2 concentrations have been determined to range from 0.5 to 5.0 s. These results indicate that the range of T_1 to be expected in tissue in vivo is about 1–20 s. In fact, given the relative inefficiency of the known non-paramagnetic relaxation mechanisms, it is suspected that T_1 in many tissues will not fall below seconds or even tens of seconds. These results are of critical importance to physiological studies using ^{129}Xe magnetic resonance spectroscopy.

Using Boltzmann polarization ^{129}Xe data have been obtained which allow estimation of T_1=38 s (±8 s, SD) for ^{129}Xe dissolved in rat blood at 293° K. (Reference 17). However, since 12 hours were required to obtain this data set, the result serves only to estimate what the normal physiological T_1 might be in vivo.

This estimate of T_1≈38 s for ^{129}Xe dissolved in rat blood at 293° K. is very encouraging. Although this result, obtained over a 12 hr period (using Boltzmann ^{129}Xe), might not be representative of physiological blood, the changes likely to occur in blood maintained at room temperature for long periods, e.g., methemoglobin formation, would tend to decrease the value observed for T_1. One can also estimate T_1 values for other model systems. The T_1 of ^{129}Xe in water has been measured at 300° K. to be 130 s. (Reference 16). ^{129}Xe exchange with protein binding sites will lower this value, (Reference 16), but the contribution from aqueous O_2 should be minimal. T_1 for ^{129}Xe in octanol, a classic membrane phase model, is 80 s. (References 16–17). Since membrane

bilayers sequester both Xe and O_2, it should be possible to use the values for Xenon and Oxygen distribution ratios, (Reference 45), between octanol and water of 20:1 and 6:1, respectively, and of the O_2 relaxivity in octanol of 0.029 $s^{-1}mM^{-1}$ at 300° K., (Reference 17), to estimate the T_1 value for ^{129}Xe in fully oxygenated membranes to be >15 s. While the actual values of T_1 in each tissue must be, and remain to be, determined, it is expected that the minimum value will fall above 15 s, a duration sufficient to enable significant accumulation of polarized ^{129}Xe in major tissues.

The unusual and extraordinary properties of hyperpolarized noble gases permit imaging of a wide variety of organs, body systems, and anatomical structures. Such structures can be imaged in live or deceased subjects, depending on application, and such subjects can include human as well as animal subjects. For example, hyperpolarized Xenon will have particular clinical importance in providing nuclear magnetic resonance imaging of neural tissue diseases, vascular plaques, compromised blood flow, tumors, as well as functional imaging of the brain's response to sensory stimuli. The properties of other noble gases will render them useful in a variety of other situations. For example, it is expected that because of its low solubility, ^3He will be of major clinical importance in imaging anatomical spaces such as lung or other artificially inflated organs.

The differential solubility of Xenon and other lipid soluble, hyperpolarizable noble gas isotopes would permit noble gas NMR differentiation between white and gray matter in brain tissue, while lipid membranes are essentially invisible to ^1H_2O MRI For example with respect to neural tissue disease, in white matter regions of the lower medulla and the spinal cord ^1H_2O MRI contrast is poor while the high lipid solubility of Xenon and other noble gas anesthetics will permit imaging of hyperpolarized isotopes. Such imaging would have diagnostic importance for patients suffering from nerve tissue demyelination. Hyperpolarized noble gas MRI would be of use for imaging of subdural hematomas as well as cystic and necrotic changes. Indications of low noble gas uptake in avascular regions would be valuable in demonstrating isodense fluid collections. (Reference 56). With respect to differentiation between tumors and infarcts, in ischemic lesions, noble gas washin/washout is delayed and blood flow is diminished, while in infarcted tissue, only the noble gas equilibrium level is diminished. In cases of multiple sclerosis ^1H_2O MRI often cannot provide useful images of plaques, while differential noble gas uptake (high in normal tissue vs. low in demyelinated plaques) would permit effective Xenon images. Similarly, in cerebral vascular and peripheral blood vessel plaques, the plaques have little or no noble gas uptake and would appear dark in a noble gas image. (Reference 57).

Images of Xenon (and other noble gas anesthetics) would also indicate cerebral, coronary and peripheral vessel defects; providing obvious indications of blood vessel constrictions and aneurysms. In particular, measurements of regional cerebral blood flow would be possible with greater exactness than is possible with other techniques. Also, study of the effects of spasms on blood flow in cases of subarachnoid hemorrhage would be rendered possible.

Functional study of brain tissue is also expected to be dramatically enhanced by the imaging of hyperpolarized noble gas anesthetics, especially Xenon, according to the invention. For example, changes in local blood flow caused by visual, tactile, and other stimuli should produce dramatic fluctuations in ^{129}Xe signal intensity. In addition, the elucidation of the precise relationships between neurological changes and psychological states has been a major goal of neurobiologists. Electroencephalography, positron emission tomography (PET) and recently, ^1H_2O MRI have been used in this field. Hyperpolarized Xenon MRI, with its high sensitivity, as exploited through fast electronics, has the potential to make huge contributions to this area. Disease states such as epilepsy, schizophrenia, depression and bipolar illness can be studied.

Clearly, hyperpolarized noble gas MRI has essentially unlimited potential application in medical settings. Hyperpolarized noble gas MRI could displace or supplement conventional MRI, and even the ubiquitous but intrusive X-ray CT scan, in at least several large areas: (1) the lung, heart, and cardiovascular systems; (2) the brain, especially since brain membrane lipids are invisible using current techniques; (3) brain function, since the ^{129}Xe signal will respond directly and strongly to metabolic changes in neural tissue.

Noble gas MRI promises to complement ^1H_2O-based imaging in a dramatic way. The near million-fold enhancement in sensitivity to noble gases enabled by hyperpolarization should result in temporal and spatial resolution in imaging superior to that achievable with ^1H_2O. In addition, the solubility of, for example, Xenon in lipids should permit imaging of organs that currently require far more intrusive techniques such as X-ray computerized tomography scanning.

The following non-limiting Examples are intended to further illustrate the present invention. In the Examples provided below, the experimental conditions were as follows unless otherwise noted: magnetic resonance spectra were obtained using a Bruker MSL 400 spectrometer equipped with a 9.4 T widebore vertical magnet, an ASPECT 3000 computer, a BVT 1000 variable temperature control unit, and employing a high-gradient Bruker micro-imaging probe and solenoidal transceiver coils of 13.3 and 20 mm diameter, operating at 110.7 MHz for ^{129}Xe and 400 MHz for ^1H. The spectrometer was not field frequency locked during the image acquisitions.

EXAMPLE 1

Xenon-Oxygen and Xenon-Oxygen-octanol "Boltzmann" imaging phantoms were prepared by standard quantitative high-vacuum gas-transfer techniques Xenon gas, enriched to 70% ^{129}Xe, was obtained from Isotec Inc., of Miamisburg, Ohio.

Image acquisition made use of a Fast-Low-Angle-SHot (FLASH) phase refocused, free-precession, fast gradient-echo imaging sequence as described by Haase et al. (Reference 58). This sampling-pulse technique was originally introduced by Look et al. (Reference 59). Standard proton microimaging gradients of 100 mT/m yielded a 50×50 mm^2 field of view for ^{129}Xe. A 128×64 encoding matrix was used, which set the spatial resolution to 0.8×0.8×8 mm^3.

FIG. 2*b* illustrates an image of a 20 mm ^{129}Xe glass phantom containing 5 atm Xe at Boltzmann equilibrium polarization (2 atm O_2 was used to reduce T_1). This image may be compared to those images in FIG. 3*c* and 3*d*. FIG. 3 illustrates the spectrum and images of a ^{129}Xe gas-octanol glass phantom containing ca. 5 atm Xe at Boltzmann equilibrium polarization (2 atm O_2 was used to reduce T_1). The observed resolution of 1×2×20 mm^3 per volumetric picture element (voxel) was achieved by accumulating 64 replicate FLASH imaging sequences over 7 min. Note that, as shown in FIG. 3*b*, the ^{129}Xe signals from the gas and octanol phases are separated by 186 ppm: this implies that the imaging gradients produce no overlap.

EXAMPLE 2

Images of hyperpolarized ^{129}Xe in glass sphere phantoms were obtained as follows. Optical pumping cells were constructed of 13–18 mm diameter Pyrex® spheres. Before filling, the cells were coated with a siliconizing agent Surfasil obtained from Pierce, of Rockford, Ill., attached to a high vacuum manifold, evacuated to $\sim 10^{-8}$ Torr, and baked at 150° C. for about 24 hours. The silicone coating apparently reduces relaxation of ^{129}Xe on the walls of the glass sphere, permitting creation of larger polarizations. The spheres were then filled with 400–1800 Torr Xe, 75 Torr N_2 and a few milligrams of Rubidium metal. Once filled with the test gas or gas/liquid, the glass cells were flame sealed.

Optical polarization was performed generally in accordance with techniques known in the art, in particular the methods of Cates et al., (Reference 35), as follows. The cells were heated to 85° C.. The entire volume of the cell was exposed to 2–4 W of 795 nm Rb D_1 laser light from a Spectra Physics 3900S Titanium-Sapphire laser, which was itself pumped by a Spectra Physics 171 Argon-Ion laser operating at 18–23 W. Both lasers were obtained from Spectra Physics of Mountain View, Calif. The laser illumination of the cells was performed in the bore of the 9.4 T magnet described above, at a field strength of 9.4 T. After 15–20 min. of optical pumping, the cells were cooled to room temperature and employed for MR experiments.

Image acquisition made use of a Fast-Low-Angle-SHot (FLASH) phase refocused, free-precession, fast gradient-echo imaging sequence as described by Haase et al. (Reference 49). This sampling-pulse technique was originally introduced by Look et al. (Reference 50). This technique takes advantage of the fact that, for small θ, the transverse projection, i.e., sin θ, allows substantial signal strength, while the loss in longitudinal projection, i.e., 1-cos θ, permits only a small loss in Z-magnetization per pulse. Standard proton microimaging gradients of 100 mT/m yielded a 50×50 mm^2 field of view for ^{129}Xe. A 128×128 encoding matrix was used, which set the spatial resolution to 0.37×0.37×1 mm^3.

FIG. 4 illustrates a series of images obtained from slices in the plane defined by the Y and Z axes through a 13 mm diameter cell containing 400 Torr of laser-polarized Xenon. The laser-polarization was performed within the bore of the 9.4 T magnet. Each image was collected in a single FLASH sequence lasting 600 msec., with 0.37×0.37×1 mm^3 resolution. FIG. 4*d* displays the variation in ^{129}Xe intensity characteristic of an image slice through a domed end of the sphere. The other slices were obtained from sections closer to the center of the spherical phantom and are more homogeneous and uniformly bright. For this experiment the ^{129}Xe polarization was estimated to be 25–30% by signal comparison to a cell of identical dimensions containing Xenon at a higher pressure but at Boltzmann polarization (illustrated in FIG. 3*b*).

EXAMPLE 3

Nuclear magnetic resonance images of mouse lungs were obtained using hyperpolarized ^{129}Xe according to the following method.

In order to deliver a quantity of hyperpolarized ^{129}Xe to a biological specimen, several obstacles must be overcome. To date, ^{129}Xe has only been successfully hyperpolarized in very pristine environments such as sealed glass cells. Such purity is essential because any paramagnetic impurities will greatly reduce the longitudinal relaxation time T_1 of the gas and thus lower the achievable polarizations. To preserve the successful sealed-cell polarization techniques and still deliver the polarized gas to an external specimen, cells equipped with thin break seals were developed. A glass delivery tube, equipped with a piston, was devised so that, once the ^{129}Xe was polarized, the cells could be sealed into the delivery tube, their break seals broken by the action of the piston, and the polarized gas freed to expand into the biological specimen.

FIG. 7 shows a delivery tube device **10** developed for the delivery of a noble gas, e.g., ^{129}Xe from a sealed cell **16** to a sample within the bore of an NMR spectrometer. The delivery device **10** includes a cylinder **12** within which a piston **14** can be controllably displaced in an axial direction. The cylinder **12** is threaded on an external surface at one end. The cylinder threads match threads on the internal surface of a control handle **22** which is rotatably attached to the piston **14**. The device also includes at least one O-ring **24, 26** providing a gas tight seal between the internal surface of the piston **14**, while permitting axial movement of the piston relative to the cylinder. At the other end of the cylinder **12**, i.e., opposite the threads adapted for receiving the control handle **22**, is sealable inlet port **20** adapted for receiving a breakable neck **18** of the sealed cell **16** containing pressurized noble gas. The inlet port **20** is sealed with a glass-sealing wax around the breakable neck **18** of the sealed cell **16** containing pressurized noble gas. The delivery device **10** also includes an outlet **28** communicating with the inlet port **20** connected to a conduit **30** to a medical sample and through which a noble gas can be delivered to the sample. The dead volume **32** in the delivery device is preferably as small as possible to minimize dilution of the noble gas as it passes from the cell **16** to the medical sample during operation of the delivery device **10**. The O-rings **24** are therefore also preferably positioned as close to the break point of the sealed neck **18** as possible.

The device **10** is preferably operated in situ, i.e., inside the NMR spectrometer used for imaging the noble gas in the sample, and is designed so that the seal of cell **16** can be broken by remote manipulation of the control handle **22**, which when rotated displaces the piston **14** toward the neck **16** until contact with neck **16** is made sufficient to break neck **16** and release the pressurized noble gas.

Mouse lungs, intact with trachea and heart were freshly excised from 30–35 g Swiss-Webster mice which had been freshly euthanized with 100 mg/kg sodium pentobarbital. The trachea was intubated with 1 mm OD Silastic medical grade tubing and the heart-lung preparation was placed in a 10 mm internal diameter glass cylinder, inserted into a 13.3 mm imaging coil and flushed with one inflation of N_2. Polarized Xenon gas was prepared as described in Example 2 except that the cells were illuminated away the bore of the 9.4 T magnet at a low field strength (approximately 10 mT). The hyperpolarized ^{129}Xe was delivered through the use of 18 mm OD Pyrex spheres provided with break-seal stems which had been sealed into a vacuum tight glass delivery tube (illustrated in FIG. 7) suspended in the bore of the magnet. The tubing from the mouse trachea was attached to the end of the delivery tube. Once the break-seal had been fractured, the 13–20 atm/cm^3 Xenon was free to expand into the lung. Gas pressures and volumes were adjusted to inflate the lung to approximately 1 atm of gas within one second, during which time only a minimal amount of relaxation of the polarization could take place.

Images were obtained using the NMR protocol described in Example 2 above. FIG. 5 presents a sequence of images illustrating the time-evolution (t=0–10 s) of the distribution

of hyperpolarized ^{129}Xe entering the lung of a heart preparation. The images represent 1.0 mm thick slices through mouse lung inflated with laser-polarized ^{129}Xe gas. The plane of the slices is perpendicular to the (absent) vertebral column (i.e., anatomical cross section). Voxel size is 0.37× 0.37×1 mm^3, and specimen diameter is 10 mm

FIG. 5*a* shows a ^{129}Xe image of lung obtained immediately after inflation (i.e., t=0 s), such that the lung is completely expanded to fill the glass cylindrical enclosure. At this point, the lung still largely contains the N_2 from the dead volume of the delivery system. Only the trachea, hints of the bronchi, and some of the lung periphery have received ^{129}Xe at this point.

FIG. 5*b* is an image obtained about 1 s later than the image in FIG. 5*a* (i.e., t=1 s). At this time the maximally inflated lung has received substantial ^{129}Xe. Both lobes of the lung can be seen with significant contrast variation and a small darker central region where the heart excludes the Xenon gas. Note that the lobes of the lung have expanded to press against the interior surface of the 10 mm diameter glass tube in which they are contained.

FIG. 5*c* is an image obtained seven seconds later than the image obtained in FIG. 5*b*, (i.e., t=8 s), showing that the lung has partially deflated. The lobes are more clearly delineated and the central heart space is more apparent. The y-axis resolution of this image is lower because it was anticipated, incorrectly, that the ^{129}Xe magnetization remaining after the image in FIG. 5*b* would necessitate the use of larger voxels and fewer slice selection pulses. Thus not all imaging parameters were optimized in acquiring these images. Optimization would likely have produce resolution between 2 and 4 times better than that achieved.

Finally, FIG. 5*d* shows a ^1H image of the same slice of the heart-lung preparation. The heart, just below center, is the primary source of intensity, while a drop of saline delineates the upper left boundary, as confirmed by visual observation of the sample.

Thus, the ^{129}Xe lung image is an excellent complement to standard proton NMR imaging. The ^{129}Xe image is clearly bright where the ^1H image is dark, and vice versa. Lung tissue is not readily seen in water proton images; only at magnified intensity does one see a faint trace of the lobes. It is believed that this phenomenon is not the result of a relative lack of protons, but is almost certainly due to the extreme local variation in bulk magnetic susceptibility at the highly complex gas-tissue interface which causes extremely short T_2 values. (See Reference 8). This is, evidently, not a problem for gas phase ^{129}Xe.

FIG. 6 shows the time variation of ^{129}Xe magnetization in the same lung as that imaged in FIG. 5 after another bolus of hyperpolarized ^{129}Xe. The decrease in ^{129}Xe magnetization following the rapid influx of ^{129}Xe into the lung is distinctly not monoexponential. The curve, decomposed into a sum of two exponentials, allows a value of T_1 of approximately 28 s to be extracted from the trailing edge of the decay. The early decrease in intensity probably reflects bulk transfer of ^{129}Xe out of the lung (deflation to resting volume) rather than magnetization decay. This is evident from the difference between the turgid lung in FIG. 5*b* (ca. 1 s after Xe release) and the lung in FIG. 5*c* (7 s later): the lobes have shrunk and the bright trachea has descended. This effect was confirmed visually using boli of N_2.

The ^{129}Xe images shown in FIG. 5 were obtained in 600 ms using a Xenon concentration of approximately 40 mM, a concentration which is tiny compared to the 80–100 M concentrations of proton typical of ^1H_2O imaging. Nonetheless the signal intensities, spatial resolution (<0.3 mm^3), and data acquisition rates all exceed those obtained in conventional clinical ^1H_2O-MRI. Moreover, the magnetization densities are so large that several images can be generated in rapid succession, allowing for real-time tracking of physiological processes.

It is believed that these images are the first reported for either Boltzmann or laser polarized ^{129}Xe. While FIG. 5 demonstrates quite clearly the power of this technique for imaging the lungs, it may turn out that ^3He which has a larger magnetic moment, longer gas phase T_1 values, (References 60–61), and which is significantly less expensive than ^{129}Xe, may be the nucleus of choice for lung imaging. However, the features of Xenon which are unmatched by the lighter noble gases, include its good solubility in non-polar solvents and its high electronic polarizability, (Reference 47), which is responsible for the extreme sensitivity of the ^{129}Xe resonance frequency and relaxation time values to environment. (References 16–17).

Such applications, however, do require that the longitudinal relaxation time of polarized ^{129}Xe be long compared to the time scales of the processes being studied. The question that immediately arises is whether the T_1 of polarized ^{129}Xe in the lung is long enough to permit transport of sufficient magnetized probe to the various tissues, and whether T_1 in these tissues will allow survival of adequate signal for imaging.

While long relaxation times can be attained by laboriously constructing pristine environments such as pumping cells (T_1>30 min), biological situations pose a marked departure from such ideal conditions. For instance, as noted above, paramagnetic O_2 in the gas phase has been shown to relax ^{129}Xe with a relaxivity of 0.0087 $s^{-1}mM^{-1}$. (Reference 18). Measurements showing that T_1≈28 s in the nitrogen flushed lung indicate that this is quite sufficient for lung imaging applications. This is demonstrated by the fact that two images, 7 seconds apart, could be acquired with a single bolus of Xenon. For the case of a live, breathing animal, we can use the O_2 relaxivity data to estimate the contribution to relaxation for the component of Oxygen contained in alveolar air (~110 Torr, 5.7 mM). For an animal breathing 40–75% Xenon and 20% O_2, we estimate T_1 to be on the order of 10–15 s, which is clearly adequate for lung imaging (FIG. 9.3*c*). Moreover, 12 s represents 5–10 blood circuits in a mouse, (Reference 62), and nearly a full circuit in a human. (Reference 63). Pulmonary blood should receive adequate concentrations of polarized ^{129}Xe.

The high ^{129}Xe polarizations attained permit the use of high-speed imaging protocols hitherto limited to ^1H_2O. We note that our field gradients and acquisition programs, conservatively chosen to match standard ^1H_2O protocols, waste both time and ^{129}Xe magnetization sampling empty voxels. Without any optimization of parameters, the contrast and resolution are already quite adequate. Future optimization of imaging parameters should easily improve upon these early images. Moreover, typical voxel sizes for human specimens, especially under the more exigent restraints of functional imaging, are 3×3×8 mm^3, or larger. (Reference 64). This represents a voxel that is 500 times larger than those displayed in FIG. 5. This represents, of course, either 500 fold more ^{129}Xe spins per voxel or the feasibility of 500 fold dilution of the ^{129}Xe for equivalent signal intensity.

Though our studies made use of relatively expensive isotopically enriched Xenon (70% ^{129}Xe), a sacrifice of a factor of only 3 in MR signal would result from the use of inexpensive natural abundance Xenon (26% ^{129}Xe).

Because the polarizations achieved through optical techniques are entirely field-independent, (References 32, 20), MR signals scale only linearly with field. Thus, MRI using laser-polarized gases can be performed at lower magnetic fields with only linear sacrifices in signal intensity (as opposed to the quadratic loss with Boltzmann polarization MR). In fact, the ratio of hyperpolarized to Boltzmann spin excess increases as magnetic field decreases; thus, in a 1 T clinical magnet the ratio is 10^6.

If the actual relaxation times in different physiological environments turn out to be close to those estimated above, the extension of ^{129}Xe imaging to other parts of the body should prove to be limitless.

APPENDIUM OF REFERENCES

1. Wyrwicz, A. M., Schofield, J. C., Tillman, P. C., Gordon, R. E. , and Martin, P. A., *Science,* 222, 428 (1983).
2. Miller, K. W., Reo, N. V., Uiterkamp, A. J. M. S., Stengle, D. P., Stengle, T. R., and Williamson, K. L., *Proc. Natl. Acad. Sci. USA,* 78, 4946 (1981).
3. Evers, A. S., Berkowitz, B. A., and d'Avignon, D. A., *Nature,* 328, 157 (1987).
4. Wyrwicz, A. M., Li, Y. -E., and Schofield, J. C., *FEBS Lett.,* 162, 334 (1983).
5. Burt, C. T., Moore, R. R., Roberts, M. F., and Brady, T. J., *Biochim. Biophys. Acta.,* 805, 375 (1984).
6. Burt, C. T., Moore, R. R., and Roberts, M. F., *J. Magn. Reson.,* 53, 163 (1983).
7. Lockhart, S. H., Cohen, Y., Yasuda, N., Kim, F., Litt, L., Eger, E. I., Chang, L. -H., and James T., *Anesthesiology,* 73, 455 (1990).
8. Mason, J., in *Multinuclear NMR,* eds. Mason, J., p 606–607, Plenum Press, New York (1987).
9. Barany, M., Spigos, D. G., Mok, E., Venkatasubramanian, P. N., Wilbur, A. C., and Langer, B. G., *Magn. Reson. Imaging,* 5, 393 (1987).
10. Fullerton, G. D., and Cameron, I. L., in *Biomedical Magnetic Resonance Imaging: Principles, Methodology, and Applications,* eds. Wehrli, F. W., Shaw, D. S., and Kneeland, J. B., p. 115–155, VCH Publishers, New York (1988).
11. Susskind, H., Atkins, H. L., Klopper, J. K., Ansari, A. N., Richards, P., and Fairchild, R. G., *Prog. Nucl. Med.,* 5, 144 (1978).
12. Susskind, H., Ellis, K. J., Atkins, H. L., Cohn, S. H., and Richards, P., *Prog. Nucl. Med.,* 5, 13 (1978).
13. Kendall, B. E., and Moseley, I. F., *J. Neuroradiology,* 8, 3 (1981).
14. Imai, A., Meyer, J. S., Kobari, M., Ichijo, M., Shinohara, T., and Oravez, W. T., *Neuroradiology,* 30, 463 (1988).
15. Yonas, H., Sekhar, L., Johnson, D. W., and Gur, D., *Neurosurgery,* 24, 368 (1989).
16. Albert, M. S., Springer, C. S., Murphy, R., and Wishnia, A., *Abs. 11th Ann. Mtg. Soc. Magn. Reson. Med.,* 2104 (1992).
17. Albert, M. S., Springer, C. S., and Wishnia, A., *Abs. 11th Ann. Mtg. Soc. Magn. Reson. Med.,* 4710 (1992).
18. Jameson, C. J., Jameson, A. K., and Hwang, J. K., *J. Chem. Phys.,* 89, 4074 (1988).
19. Carver, T. R. *Science,* 141, 599 (1963).
20. Happer, W., Miron, E., Schaefer, S., Schreiber, D., van Wijngaarder, W. A., and Zeng, X., *Phys. Rev. A,* 29, 3092 (1984).
21. Wagshul, M. E. and Chupp, T. E., *Phys. Rev. A,* 40, 4447 (1989).
22. Wagshul, M. E., Thesis, The Department of Physics, Harvard University (1991).
23. Grover, B. C., *Phys. Rev. Lett.,* 40, 391 (1978).
24. Schaefer, S. R., Cates, G. D., Chien, T. -R., Gonatas, D., Happer, W., and Walker, T. G., *Phys. Rev. A.,* 39, 5613 (1989).
25. Schaefer, S. R., Cates, G. D., and Happer, W., *Phys. Rev. A.,* 41, 6063 (1990).
26. Schearer, L. D., in *Phys. Rev. Lett.,* 21, 660 (1968).
27. Schearer, L. D., in *Phys. Rev.,* 188, 505 (1969).
28. Schearer, L. D., in *Phys. Rev.,* 180, 83 (1969).
29. Colegrove, F. D., Schearer, L. D., and Walters, G. K., *Phys. Rev.,* 132, 2561 (1963).
30. Hadeishi, T., and Liu, C. -H., *Phys. Rev. Lett.,* 19, 211 (1967).
31. Schearer, L. D., *Phys. Lett.,* 28A, 660 (1969).
32. Cates, G. D., Benton, D. R., Gatzke, M., Happer, W., Hasson, K. C., and Newbury, N. R., *Phys. Rev. Lett.,* 65, 2591 (1990).
33. Gatzke, M., Cates, G. D., Driehuys, B., Fox, D., Happer, W., and Saam, B., *Phys. Rev. Lett.,* 70, 690 (1993) .
34. Bhaskar, N. D., Happer, W., and McClelland, T., *Phys. Rev. Lett.,* 49, 25 (1982).
35. Cates, G. D., Fitzgerald, R. J., Barton, A. S., Bogorad, P., Gatzke, M., Newbury, N. R., and Saam, B., *Phys. Rev. A.,* 45, 4631 (1992).
36. Raftery, D., Long, H., Meersmann, T., Grandinetti, P. J., Reven, L., and Pines, A., *Phys. Rev. Lett.,* 66 584 (1991)
37. Raftery, D., Long, H., Reven, L., Tang, P., and Pines, A., *Chem. Phys. Lett.,* 191, 385 (1992).
38. Long, H. W. Gaede, H. C., Shore, J., Reven, L., Bowers, C. R., Kritzenberger, J., Pietrass, T., and Pines, A., *J. Am. Chem. Soc.,* 115, 8491 (1993).
39. Rinck et al., *An Introduction to Magnetic Resonance in Medicine* (1990).
40. Stark et al., eds., *Magnetic Resonance Imaging,* Vol. 1, 2d ed. (1992).
41. Hunt, E. R., and Carr, H. Y., *Phys. Rev.,* 130 , 2302 (1963).
42. Tilton, R. F., and Kuntz, I. D., *Biochemistry,* 21, 6850 (1982).
43. Diehl, P., and Jokisaari, J., *J. Magn. Reson.,* 88, 669 (1990).
44. Cullin, S. C., and Gross, E. G., *Science,* 113, 580 (1951).
45. Wilcock, R. J., Battino, R., Danforth, W. F., and Wilhelm, E., *J. Chem. Thermodyn.,* 10, 317 (1978).
46. Blumgart, H. L., and Weiss, S., *J. Clin. Invest.,* 4, 339–425 (1927).
47. Pollack, G. L., Himm, J. F., and Enyeart, J. J., *J. Chem. Phys.,* 81, 3239 (1984).
48. Wishnia, A., *Biochemistry,* 8, 5064 (1969).
49. Bouchiat M. A, Carver T. R., and Varnum C. M., *Phys. Rev. Lett.,* 5, 373 (1960).
50. Zeng, X., Wu, Z., Call, T., Miron, E., Schreiber, D., and Happer, W., *Phys. Rev. A,* 31, 260 (1985).
51. Laloe, F., Nacher, P. J., Leduc, M., and Schearer, L. D., *AIP. Conf. Proc.* #131 (Workshop on Polarized ^3He Beams and Targets) (1984).
52. Ernst et al., *Principles of Nuclear Magnetic Resonance in One and Two Dimensions* (1987).
53. Wehrli, S. W., et al., eds., *Biomedical Magnetic Resonance Imaging* (1988).
54. Schoenborn, B. P., *Nature,* 208, 760 (1965).
55. Moschos, A., and Reisse, J., *J. Mag. Reson.,* 95, 603 (1991).
56. Yonas, H., Laligam, S., Johnson, D. W., and Gur, D., *Neurosurgery,* 24, 368 (1989).
57. Kendall, B. E., and Moseley, I. F., *J. Neuroradiology,* 8, 3 (1981).

58. Haase, A., Frahm, J., Matthaei, D., Hanicke, W., and Merboldt, K. D., *J. Magn. Reson,* 67, 217 (1986).
59. Look, D. C., and Locker, D. R., *Rev. Sci. Instrum.,* 41, 250–251 (1970).
60. Norberg, R. E., in *Rare Gas Solids,* eds. Hohler, G., Springer-Verlag, New York (1984).
61. Yen, W. M., and Norberg, R. E., *Phys. Rev.,* 131, 269 (1963).
62. Kaplan, H. M , Brewer, N. R., and Blair, W. H., in *The Mouse in Biomedical Research,* eds, Foster, H. L., Small, J. D., and Fox, J. G., p. 248–278, Academic Press, New York (1983).
63. Knudsen, G. M., Pettigrew, K. D., Patlak, C. S., and Paulson, O. B., *Am. J. Physiol., In Press.*
64. Kanal, E., and Wehrli, F. W., in *Biomedical Magnetic Imaging,* eds. Wehrli, F. W., Shaw, D., and Kneeland, J. B., p. 47–112, VCH publishers, New York (1988).

While there have been described what are presently believed to be the preferred embodiments of the invention, those skilled in the art will realize that changes and modifications may be made thereto without departing from the spirit of the invention, and it is intended to claim all such changes and modifications as fall within the true scope of the invention.

We claim:

1. A method of nuclear magnetic resonance (NMR) imaging, which comprises the steps of:

a) administering hyperpolarized noble gas to a human or animal subject;

b) detecting a spatial distribution of said hyperpolarized noble gas in said subject by NMR; and

c) generating a representation of said spatial distribution of said hyperpolarized noble gas.

2. The method of claim 1, wherein said noble gas is selected from the group consisting of Helium-3, Neon-21, Krypton-83, Xenon-129, Xenon-131, and mixtures thereof.

3. The method of claim 2, wherein said noble gas includes Xenon-129.

4. The method of claim 2, wherein said noble gas includes Helium-3.

5. The method of claim 2 wherein said noble gas includes Xenon-129 and Helium-3.

6. The method of claim 1, further comprising the step of hyperpolarizing said noble gas prior to said administering step.

7. The method of claim 6, wherein said hyperpolarizing step comprises hyperpolarizing said noble gas through spin exchange with an alkali metal.

8. The method of claim 6, wherein said hyperpolarizing step comprises hyperpolarizing said noble gas through metastability exchange.

9. The method of claim 7, wherein said alkali metal is selected from the group consisting of Sodium-23, Potassium-39, Cesium-133, Rubidium-85, and Rubidium-87.

10. The method of claim 9, wherein said alkali metal is selected from the group comprising Rubidium-85 and Rubidium-87.

11. The method of claim 1, wherein said detecting step further comprises detecting said spatial distribution of said noble gas along at least one physical dimension.

12. The method of claim 11, wherein said detecting step further comprises detecting said spatial distribution of said noble gas along two physical dimensions.

13. The method of claim 11, wherein said detecting step further comprises detecting said spatial distribution of said noble gas along three physical dimensions.

14. The method of claim 1, wherein said representation comprises a visual representation.

15. The method of claim 1, wherein said detecting step precedes said generating step.

16. The method of claim 1, wherein said detecting step and said generating step are substantially simultaneous.

17. The method of claim 1, wherein said generating step includes generating said representation from NMR parametric data.

18. The method of claim 17, wherein said NMR parametric data includes data computationally derived from at least one physically measurable NMR parameter selected from the group consisting of chemical shift, T_1 relaxation, T_2 relaxation, and $T_{1\rho}$ relaxation.

19. The method of claim 1, wherein said noble gas is distributed in at least one physical phase in said subject.

20. The method of claim 19, wherein said noble gas is distributed in a gas in said subject.

21. The method of claim 19, wherein said noble gas is distributed in a liquid in said subject.

22. The method of claim 19, wherein said noble gas is distributed in a solid in said subject.

23. The method of claim 22, wherein said noble gas is distributed on a solid surface in said subject.

24. The method of claim 19, wherein said noble gas is distributed in at least two physical phases in said subject.

25. The method of claim 1, wherein said noble gas is distributed in an organ or body system of said human or animal subject.

26. The method of claim 25, wherein said noble gas is distributed in lung tissue of said human or animal subject.

27. The method of claim 25, wherein said noble gas is distributed in nervous tissue of said human or animal subject.

28. The method of claim 27, wherein said noble gas is distributed in brain tissue of said human or animal subject.

29. The method of claim 1, wherein said noble gas is distributed in an anatomical space of said human or animal subject.

30. The method of claim 29, wherein said anatomical space comprises lung space.

31. The method of claim 29, wherein said anatomical space comprises gastrointestinal tract space.

32. The method of claim 1, wherein said noble gas administering step comprises administering said noble gas in a gaseous form.

33. The method of claim 32, wherein said noble gas administering step comprises administering said noble gas to said human or animal subject by passive or active inhalation.

34. The method of claim 1, wherein said noble gas administering step comprises administering said noble gas to said human or animal subject included in a liquid composition.

35. The method of claim 34, wherein said noble gas administering step comprises administering said noble gas by parenteral injection.

36. The method of claim 35, wherein said noble gas administering step comprises administering said noble gas by intravenous injection.

37. The method of claim 36, wherein said noble gas administering step further comprises introducing said noble gas into blood and intravenously injecting the noble gas-containing blood into said human or animal subject.

38. The method of claim 1, wherein said representation represents at least one spatial dimension of said noble gas spatial distribution.

39. The method of claim 38, wherein said representation represents two spatial dimensions of said noble gas spatial distribution.

40. The method of claim 38, wherein said representation represents three spatial dimension of said noble gas spatial distribution.

41. The method of claim 38, wherein said representation further represents at least one spatial dimension of said noble gas spatial distribution as a function of time.

42. A method of performing nuclear magnetic resonance (NMR) imaging of a human or animal subject, which comprises the steps of:

a) administering to said subject an imageable amount of a hyperpolarized noble gas;

b) generating radio frequency signals from the hyperpolarized noble gas by means of a nuclear magnetic resonance imaging spectrometer;

c) detecting radio-frequency signals derived from nuclear magnetic resonance of the hyperpolarized noble gas;

d) processing said radio frequency signals to provide an NMR parameter data set as a function of spatial distribution of the hyperpolarized noble gas; and

e) further processing the NMR parameter data set to derive a representation corresponding to at least one spatial dimension of the spatial distribution of the hyperpolarized noble gas.

43. The method of claim 42, wherein said administering step further comprises administering a gas composition to said subject.

44. The method of claim 43, wherein said administering step further comprises passive or active inhalation of said gas composition by said subject.

45. The method of claim 43, wherein said administering step further comprises administering said noble gas as at least one bolus.

46. The method of claim 43, wherein said administering step further comprises administering said noble gas continuously during the generating and detecting steps.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,545,396
DATED : August 13, 1996
INVENTOR(S) : Albert et al.

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

Column 3, Line 24,	now reads "^{129}Xe but", should read --^{129}Xe, but--;
Column 7, Line 26,	now reads "(References 5, 47-48).", should read --(References 45, 47-48).--;
Column 9, Line 59,	now reads "(e.g. 795 nm for Rb)", should read --(e.g. 795 nm for Rb).--;
Column 10, Line 33,	now reads "^{15}Rb", should read --^{85}Rb--;
Column 15, Line 12,	now reads "The value of T_1", should read --The value of T_1--;
Column 16, Line 55,	now reads "estimate of $T_1 \approx 38$ s", should read --estimate of $T_1 \approx 38$ s--;
Column 17, Line 30,	now reads "^1H_2O MRI For", should read --^1H_2O MRI. For--;

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,545,396
DATED : August 13, 1996
INVENTOR(S) : Albert et al.

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

Column 22, Line 10, now reads "may turn out that ^{3}He which", should read --may turn out that ^{3}He, which--;

Column 22, Line 11, now reads "phase T_l values", should read --phase T_1 values-- and

Column 24, Line 25-26 now reads "66 584 (1991)"; should read --66, 584 (1991).--

Signed and Sealed this
Twenty-fourth Day of December, 1996

Attest:

BRUCE LEHMAN

Attesting Officer *Commissioner of Patents and Trademarks*

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,545,396
DATED : August 13, 1996
INVENTOR(S) : Albert et al.

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

Column 1, lines 4-6 now reads "This invention was made with government support under grant numbers 88-0165 and F49620-92-J-0211, awarded by the Air Force Office of Scientific Research.", but should read--This invention was made with government support under grant number MCB-9307654, awarded by the National Science Foundation, and grant numbers 88-0165 and F49620-92-J-0211, awarded by the Air Force Office of Scientific Research.--.

This certificate supersedes Certificate of Correction Issued Dec. 24, 1996.

Signed and Sealed this

Eighteenth Day of February, 1997

Attest:

BRUCE LEHMAN

Attesting Officer *Commissioner of Patents and Trademarks*